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METHOD FOR EVALUATING DNA PROBES POSITION ON SUBSTRATE

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BACKGROUND OF THE INVENTION

(1) Field of the Invention

This Invention concerns a DNA array and an analytical method of stress using said DNA array for the simple evaluation of degrees of stress. This Invention also concerns a method of evaluation of expression of gene groups related to certain diseases, not limiting to stress, by positioning oligonucleotides on substrate based on degree of correlation.

(2) Description of the Related Art

Increases in diseases associated with life style and atopic allergy are one of the factors that are responsible for the increase in today's medical burden to the nation. Reported also are increases in the numbers of suicides, lowering age of criminals and increases in patients with post-traumatic stress disorder (PTSD). Medical experts agree that stress play a role in background or life style-associated diseases, allergy, suicide, crime and PTSD.

Stress is defined as a reaction of the living body to sudden invasion, both as specific reaction to each invasion and as generic non-specific reaction, which has a fixed pattern regardless of the type of invasion. Stress-causing stimuli, or stressor, include abnormal temperature, burn, inflammation, immune

reaction, noise, electric shock, ultraviolet light, bacterial toxin, bacteria, virus, operation, exercise, pain stimulus, physical restrain, hypoxia, hypoglycemia, ischemia, tests, interpersonal friction, deaths of relatives, loneliness, broken heart, despair, disappointment, social unrest, war, terrorism and earth quake. With advancement in knowledge of the maintenance mechanism of bodily homeostasis, it has become clear that there is a close relationship between abnormalities of the three major regulatory mechanisms of the body, the nerve, endocrine and immune system, and stress.

In conventional oligonucleotide array, it is decided first which genes are placed on chips, and then, according to the order, such as alphabetical order, designated to genes, genes are placed on a plate, such as a 96-well plate, using a spotter with several needles. In this method, although genes are lined up systematically, a step is required at the actual evaluation to confirm the positions of genes by consulting address information on files and images that show where and which genes are placed.

However, no medicophysiological diagnostic method has been developed by which the degree of stress can be evaluated objectively. For instance, blood concentrations of stress hormones, such as catecholamine and adrenocortical hormone, vary greatly among individuals and change with time. In other

words, blood concentrations of stress hormones do not change uniformly in response to stress stimuli, and are known to be insufficient to be used for evaluation of degree of stress. In addition, it is extremely difficult to evaluate bodily reactions only by measuring these limited stress hormones because stress is the reaction of complex systems, requiring multilateral evaluations. Stress also is studied in the field of social psychology. Psychological tests in the form of interviews or questionnaires have been developed to evaluate degree of stress. However, it cannot be said that psychological tests substantiate sufficiently physiological reaction of the body. That is to say, currently, there scarcely are methods for objective evaluation of stress of individual persons. However, stress is an important phenomenon that is related to abnormalities of the automatic nervous system, endocrine and immune, gastric ulcer, acute lesions of gastric mucus, mental diseases and reproductive dysfunction. If it is possible to evaluate degree of stress readily at not only specialty medical organizations but also general practitioners, health facilities at business and school and health screening centers, it is a useful measure, as feedback can be implemented in daily life at home, workplace and school. From that standpoint, development of diagnostic instruments is sought that can determine the degree of stress.

The objective of this Invention is to provide

a diagnostic method, specifically, oligonucleotide array, by which degrees of stress can be determined readily and at low cost. In particular, this Invention aims at minimizing the number of DNA fragments placed on the array by specifying groups of genes, which are imperative in determination of degrees of stress, and at providing an array for stress analysis with high reproducibility and reliability. This Invention also aims at instant evaluation of the correlation between genes that are related a certain disease by devising regulations in how genes are arranged.

SUMMARY OF THE INVENTION

As mentioned above, stress is the complex reaction in which various organs, such as the nervous, endocrine and immune systems, play roles and must be evaluated from many angles. Expressed at the gene level, stress reaction, which is a phenomenon with complex sources, occurs when the on-and-off switches of groups of genes related to stress are turned on, the volume of stress-related protein increases or decreases. The body mechanism is thought to be regulated according to the balance in activities of the whole protein. In other words, abnormalities of the on-and-off mechanism in stress-related gene groups induce the abnormalities of the balance in protein activities, resulting in the abnormalities of regulation of body mechanism, or occurrence of stress. The

switching on and off of genes is controlled, for example, by increases or decreases in the level of gene expression. The level of gene expression can be measured using the level of messenger RNA or the level of protein as an index. With techniques currently available, the measurement can be performed extremely easily using the level of messenger RNA as an index rather than using the level of protein. Therefore, stress is evaluated easily by observing the increase or decrease in the level of expression of messenger RNA of several stress-related genes. DNA array (also called oligonucleotide array) developed recently is the most suitable for this purpose.

Here, the state of expression is explained in detail. The state of expression is one of genotype, and expression in the term "the state of expression' means the state, where the region of genes on DNA is transcripted on to RNA, or protein is translated through transcribed RNA. The state in the term "the state of expression" means a row of "n" pieces of genes, or gene 1, gene 2, so forth, ending with gene "n". When ON indicates that expression takes place and OFF indicated that expression does not take place, there is a row of (ON or OFF), (ON or OFF), repeating "n" times; this is called "state". When with "n" pieces of genes, UP indicates increased level of RNA transcription, EVEN indicate unchanged, and DOWN indicates decreased, there is a row of (UP, EVEN or

DOWN), (UP, EVEN or DOWN), repeating "n" times; this is called "state". The correlation between 2 genes, any 2 among "n" pieces of genes, is "state", that is to say, when the intensity of measurement signal of gene i is X and the intensity of measurement signal of gene j is Y, and mean of X and Y in N times of experiments are m(X) and m(Y), and standard deviations are S(X) and S(Y), respectively, the matrix of the correlation coefficient "r", or r(i,j) is "state". Correlation coefficient can be expressed, for example, in the following equation (1).

$$r(i,j)=\sum_{k=1}^{N}(X-m(X))/S(X)\times(Y-m(Y))/S(Y))/(N-1) \qquad (1)$$

Changes in the above-mentioned the state expression, that is, changes in genotype induce changes in phenotype. Phenotype means phenomenon that can be observed from outside by some means. Phenotype, for example is disease or symptoms and sites of the body where symptoms appear. Disease is a pathophysiological state that physicians can diagnose by experience, such as diabetes mellitus and cancer. Symptom is a phenomenon persons feel subjectively, such as headache and abdominal pain. Symptom also is different from normal values that can be detected by test apparatus; for example, neutral fat is above the standard value in obesity. Included also in phenotype are some things that can be observed from outside by some means,

excluding difference in cell configuration and in velocity of cell growth.

DNA array (oligonucleotide array) comprises plural DNA fragments (oligonucleotide) that are fixed on substrate. Each nucleotide corresponds to different genes. In measurement, complementary DNA (cDNA) fragments are synthesized in reaction with reverse transcriptase using messenger RNA as a template. At the time of the reaction with reverse transcriptase, an appropriate label binds with cDNA fragments or is incorporated when a strand is extended for labeling of cDNA (hereinafter, such cDNA is called labeled cDNA). Complementary binding takes place between oligonucleotide fixed on substrate and labeled cDNA fragments. Coordinates on substrate on which oligonucleotide are fixed, all differ. If it is known beforehand which oligonucleotide is fixed on which coordinates, increases or decreases in messenger RNA can be measured simultaneously in plural numbers of genes.

In order to achieve the objective that degree of stress is evaluated using oligonucleotide, this Inventors investigated and found that it is necessary to place on the same array many genes, or at least 30 or more different genes, and more desirably several hundred DNA fragments (Oligonucleotide fragments; probe DNA). Those genes are; (1) internal; and external standard genes for proofreading (housekeeping genes),

(2) stress-related genes such as heat shock protein (HSP) and hormone genes such as sex hormone that decreases under stress, (3) cytokine genes that induce immune response and inflammatory reaction, (4) genes that induce cell death, (5) genes related to anti-inflammation and wound healing, and genes related to cell growth inhibition, such as glucocorticoid, TGFβ and FGF, (6) transcription factor and signaling molecules related to immune response, (7) transcription factor and signaling molecules related to induction of cytokine, which causes cell injury, (8) transcription factor and signaling molecules related to growth inhibition, and (9) transcription factor and signaling molecules related to stress response. The above (1) to (5) are functional genes that govern specific functions in the body, and (6) to (9) are signal transfer genes that govern transmission of signals between functional genes.

This Inventors also found that by positioning DNA probes that are to be fixed on substrate according to gene classification of the above (1) to (9), results of measurement of DNA array can be understood and evaluated immediately. In addition, this Inventors found that by using leukocytes that are relatively easily collected from subjects, for whom messenger RNA is tested, as specimens for tests, degrees of stress can be easily evaluated. Thus, this Invention was completed. Concrete means to solve problems are

explained below.

This Invention is an array on which plural oligonucleotides with different base sequences are fixed at known, different positions on a support medium, and the oligonucleotide array is characterized by the fact that the said oligonucleotides are those of genes mentioned in the above (1) to (9) or strands of complementary sequences on the said genes, and the base sequence of said oligonucleotides comprises bases that number at least 20 or more.

An oligonucleotide array of this Invention also is characterized by the fact that nucleotides are those of genes related to mediating factors that intermediate 3 parties of the endocrine, immune and nervous systems that are known to work in coordination in stress reaction, or those of strands of complementary sequences, and the base sequence of said oligonucleotides comprises bases numbering at least 20 or more. Examples of said mediating factors include corticotropin releasing hormone (CRH) and cytokine.

In addition, an oligonucleotide array of this Invention is characterized by the fact that oligonucleotides fixed on the same support medium have the base sequence comprising bases that number at least 20 or more, and consist of gene groups related to 2 or more different signal transfer pathways or strand groups of complementary sequences on said gene groups. Said gene groups comprise at least 2 or more types of

genes that code intracellular signal transfer related protein groups that lie between cell membrane receptors and intranuclear receptors and transcription factors that are on the same signal transfer pathway.

Futhermore, this Invention is a gene expression analytical method using two oligonucleotide arrays. Using the first oligonucleotide array with plural oligonucleotides with different base sequences that are fixed on a support medium, gene expression analysis is conducted comprehensively to select gene groups that show changes in the level of expression and gene groups related to said gene groups. The second oligonucleotide array is made of oligonucleotides of the above selected gene groups, related gene groups and strands of complementary sequences on said selected gene groups and related gene groups. Said oligonucleotides have the base sequence comprising bases that number 20 or more and are fixed on a support medium. Said second oligonucleotide array also is used for gene expression analysis.

This invention was completed using the investigation results on stress response mentioned above. By using the oligonucleotide array of this invention, it is possible to easily evaluate the degree of disorder, malfunction, symptom (stress) judging from not only each gene but also focusing on the change of balance among the nervous system, endocrine system and immune system. Particularly, by arranging each gene on

the substrate while taking into account two axes such as "life and death" and "inflammation and anti-inflammation", intuitive evaluation of the results is possible. Also, since the oligonucleotide probes on the array of this invention are narrowed down to those that have a deep relationship with stress response, the number of oligonucleotide types to be used as probes for the array are greatly reduced, thus allowing to reduce the price. Furthermore, by fixing a single type of oligonucleotide in several positions as a probe, the signal intensity of multiple positions can be averaged to increase reliability. Also, by making a rule for arranging the gene groups, relationships between genes related to a certain disorder can be evaluated at a glance.

Other objects, features and advantages of the invention will become apparent from the following description of the embodiments of the invention taken in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 illustrates DNA probe position on substrate (Example 1).

FIGURE 2 illustrates DNA probe position on substrate (Example 2).

FIGURE 3 illustrates an example of stress evaluation.

FIGURE 4 illustrates an example of general

structure of DNA chip.

FIGURE 5 is an example of positioning rule.

FIGURE 6 illustrates an example of plural positioning on one DNA chip substrate.

FIGURE 7 illustrates an example of correlation score of genes.

FIGURE 8 illustrates an example of intergenic pathway.

FIGURE 9 illustrates an example of DNA chip making kit.

FIGURE 10(A) illustrates control scatter plot.

FIGURE 10(B) illustrates a patient's scatter plot.

FIGURE 11 illustrates an example of positioning rule.

FIGURE 12 illustrates fluorescence patterns of control A to E.

FIGURE 13 illustrates a patient's fluorescence pattern.

FIGURE 14 illustrates a flow chart of measurement using DNA chip.

FIGURE 15 illustrates an outline of DNA chip making using gene positioning in Bioinformatics.

FIGURE 16 illustrates a flow chart of DNA chip making using gene positioning in Bioinformatics.

FIGURE 17 illustrates an outline of DNA chip making using gene groups positioning based on experi-

mental results.

FIGURE 18 illustrates a flow chart of DNA chip making using gene groups positioning based on experimental results.

In the drawings, numerals represent the following:

1. Substrate, 2. Probe DNA fixation region, 11. Probe DNA of housekeeping genes, 12. Probe DNA of stress-tolerance and survival-related genes and hormones, 13. Probe DNA of inflammation-, immune response-, and cell proliferation-related genes, 14. Probe DNA of apoptosis and cell death-inducing genes, 15. Probe DNA of gene related to anti-inflammation, wound-healing, and cell growth inhibition, 16. Probe DNA of immune response related transcription factors and signaling molecules, 17. Probe DNA of cytokine inductive transcription factors and signaling molecules, 18. Probe DNA of cell growth inhibition related transcription factors and signaling molecules, 19. Probe DNA of stress response related transcription factors and signaling molecules, 20. Fluorescence detector, 21. DNA probe, 22. Fluorescence labeled gene, 23. Supporter, 24. Example of probe positioning according to expression pattern, 25. Gene, 26. Correlation score, 27. Gene, 28. Inter-gene pass way, 29. Reagent, 30. Spotter, 31. Computer for controlling the spotter, 32. Chip (being made), 33. Chip (finished), 34. Fluorescence detector, 35. Computer for controlling the fluorescence detector, 36.

Positioning information file, 37. Public database, 38. In-house database, 39. Network connected computer, 40, Probe stock, 41. Automatic dispenser, 42. Probe to be spotted, 46. Experimental results, 47. Computer for experimental data analysis.

PREFERRED EMBODIMENT OF THE INVENTION

Figure 4 illustrates a general structure of DNA chip. Figure 14 illustrates a flow chart of measurement using DNA chip. First, DNA probes (21) are fixed on to a support medium (23). Gene fragments extracted from samples obtained from subjects of measurement are labeled with fluorescent label, etc. The fluorescent-labeled gene (22) and DNA probes (21) are hybridized. Then, fluorescence light originated from fluorescent label is detected by a detector (20). Detection demonstrates the level of fluorescent-labeled gene (22) that were hybridized with each of DNA probes (21). This is called gene expression profile.

Oligonucleotide, that is, DNA probe, is classified according to P value, FDD and SVD. The P value is a value called in statistics as significant probability, which expresses degrees of dissociation of statistics from null hypothesis in hypothesis testing. The P value is expressed between 0 and 1. The smaller the figure is the larger the dissociation is. The null hypothesis in the Specification of this application is defined as "there is no difference in the level of

expression between gene A originating RNA and gene B originating RNA." When P is 0, it means that gene A originating RNA differs from gene B originating RNA, and when P is 1, it means that gene A originating RNA is the same as gene B originating RNA. The P value can be obtained in, for example, parametric tests such as t-test and F-test or non-parametric tests such as Wilcoxon test.

Differential display is one of methods of detecting the difference in messenger RNA that expresses in cells under different conditions. The principle of the method is that messenger RNA that is reverse transcribed using oligo dT primer is combined with various primers. The combinations are amplified in PCR for comparison of band patterns in electrophoresis in each sample. When fluorescent labeling is used for signal detection, it is called fluorescent differential display (FDD). Messenger RNA that expresses can be either known or unknown.

Support vector machine (SVM) is a method based on machine learning used for classification of hand-written letters and images, and one of methods used to classify given data into plural categories. SVM is an algorithm with which differences among messenger RNA expressing in cells under different conditions are classified. Thus, SVM is an algorithm of classification that belongs to supervised methods. Similar methods include nearest neighbor, discriminant

analysis, neural network and classification tree.boosting bagging. Although the Specification of this application mentions SMV as the typical example, any classification methods can be used.

For example in order to evaluate degrees of stress, it is necessary to conduct highly accurate analysis of the mechanism of function of stress response. It is clearly avoided that DNA fragments that should have complementary binding with one kind of genes bind with other genes (cross hybridization). It becomes progressively difficult, as the number of genes that are fixed on a piece of array increases. Consequently, it is extremely difficult to eliminate completely cross hybridization among five-thousands to several ten-thousand genes on one DNA-array for detection. It became clear in investigation on sequence homology based on blast algorithm that when the base length of DNA fragments used as probes is not more than 1,000 bases, it is desirable to place less than 1,000 to 1,500 kinds of genes on one array. Therefore, if the purpose of use of DNA array is to elucidate the mechanism of action of stress response, it is desirable to collect the least possible number of genes that are related to the mechanism of action of stress response and use only these genes for array. It is not desirable to place on array genes that are not related to stress response, which will result in increases in cost of making probes, leading to eventual

increases in cost of oligonucleotides. In this Invention, the number of kinds of oligonucleotides used as probes on array can be restricted, any one kind of oligonucleotides can be fixed as probes at plural positions. Signal intensity can be obtained from plural positions, increasing reliability.

Concrete examples of positioning methods of gene groups are explained below.

1. Positioning methods of gene groups using Bioinformatics.

1) According to gene functions (Classification No. 1)

For example, gene groups are positioned as shown in FIGURES 1 and 2 in the Specification of this application. No. 11 indicates internal and external standard genes for proofreading (housekeeping genes), No. 12 stress and survival related genes and hormone genes, No. 13 inflammation, immune response, cell proliferation related genes, No. 14 apoptosis and cell death related genes, No. 15 anti-inflammation, wound-healing, cell growth inhibition related genes, No. 16 immune response related transcription factor signaling molecules, No. 17 cytokine inductive transcription factor, signaling molecules, No. 18 cell growth inhibition related transcription factor, signaling molecules, and No. 19 stress response related transcription factor, signaling molecules. FIGURE 1 illustrates an example in which the above 11 to 19 are positioned at 9 fixed regions. FIGURE 2 illustrates an

example in which 11, 12 and 19, 13 and 16, 14 and 17, and 15 and 18 are positioned at 5 fixed regions.

Classification of genes into any among 11 to 19 is decided based on terminology defined in the ontology database constructed by the International Ontology Consortium (http://www.geneontology.org/). Gene related ontology can be searched on PubGene (http://www.pubgene.org), which is one of publicly offered ontology database, or Gene Ontology (GO). The PubGene database connects gene with ontology through textual analysis of Medline, OMIM, etc. (refer to Tor-Kristian Jenssen et al. A literature network of human genes for high- throughput analysis of gene expression. Nature Genetics, vol.28, pp21-28). In PubGene classification, HSPA1A, for example, which is a heat shock protein (HSP), is closely associated with Heat shock protein (GO No. 0003773) in the Functional Annotation and with transcription (GO No. 006350) and immune response (GO No. 0006955) in the Cell Process Annotation. Another HSP, HSPA1B, is classified to Heat shock protein (GO No. 0003773) in the Functional Annotation and apoptosis (GO No. 0006915) in the Cell Process Annotation. Therefore, according to the Functional Annotation in PubGene, for example, both HSPA1A and HSPA1B belong to the same stress related gene, that is, heat stress protein. The two are classified to No. 12 Stress and survival-related genes and hormone genes. According to the Cell Process

Annotation in PubGene, on the other hand, HSPA1A belongs to No. 13 Immune response related genes, and HSPA1B to No. 14 Apoptosis and cell death related genes. Ontology in the Functional Annotation and Cell Process Annotation in PubGene is listed in the order of scores. Therefore, ontology with the largest score or several numbers of ontology with relatively large scores are selected for classification. Along with PubGene, any tool or database can be used to search ontology based on gene names.

2) Gene positioning within fixation regions (Classification No. 2)

The final positioning of genes that are distributed on fixation regions in the above 1) is decided according to any one or the combination of two or more of the following information; (1) gene correlation scores obtained through database, (2) information on pairing of ligand and receptor, (3) information on protein-protein interaction, and (4) information on gene pathway. The list of genes contained at each fixation region is obtained in Classification No. 1. Genes on the list are sorted out in the order of gene names (or gene symbol names) or put in order impromptu. For example, gene A on the top of list is fixed at the pre-determined position, such as at the corner or center of its fixation region. Then, genes that have strong correlation with gene A are sought. Supposing that gene B and gene C have strong correlation with

gene A, then these two genes are positioned next to gene A. Gene B and gene C whose positions have been decided are eliminated from the list. Gene D, which is now at the top of the list, is positioned where genes A, B and C are not positioned. In the same manner as above, Gene E and gene F that have strong correlation with gene D are sought and positioned next to gene D. By repeating the process, genes with strong correlation with each other gather closely and form clusters within each fixation region. Methods of how to search for genes with strong correlations with each other are explained below.

In the method (1) above, it is regarded that the more frequently the two genes appear in the same sentence of the same database, the stronger the correlation between two genes is. The correlation score can be obtained, for example, by looking up PubGene database (refer to Tor-Kristian Jenssen et al. A Nature Genetics. Vol.28, pp21-28.). FIGURE 7 illustrates an example. Circles in FIGURE indicate genes, lines connecting circles the presence of correlation between genes, and numbers along lines the correlation scores. The correlation scores in FIGURE 7 indicate the frequencies in which two genes connected with a line are mentioned in the same abstract in MEDLINE. Six genes that have strong correlations with ADPRT at the center of FIGURE 7 are TP53, CFTR, EEF2, FRA1H, SP1 and ADF. Every one of 6 genes has a

correlation score 1. When plural genes have the same correlation scores, genes are sorted, for example, in the alphabetical order and positioned around ADPRT accordingly. When the correlation scores differ, genes are positioned in the order of higher scores. The database used in PubGene is MEDILINE and OMIM by the American NCBI. Database in other references can also be used.

Positioning based on the above (2) information on pairing of ligand and receptor means that genes which proteins have a relationship of ligand and receptor are positioned adjacent to each other, for example insulin-like growth factor 1 (IGF1) and insulin-like growth factor 1 receptor (IGF1R) or insulin (INS) and insulin receptor (INSR) are positioned adjacent to each other.

Positioning based on the above (3) information on protein-protein interaction means that positioning of genes are decided according to protein interaction databases such as, for example, UCLA DIP (Database of Interacting Proteins by University California Los Angeles, USA, refer to I.Xenarios et al. DIP: the database of interacting proteins. Nucleic Acid Research. Vol.28, pp.289-291, 2000). In database of interacting proteins, proteins that interact each other are connected with lines as illustrated in FIGURE 7. The intensity of interaction can be based on bonding strength of molecules, which can be indicated with, for

example, dissociation constant obtained in experiments. The higher the bonding strength is, the greater the interaction intensity is. In addition, the interaction intensity that is confirmed in plural, or more than 2, experiments can be regarded stronger than that confirmed in just 1 experiment. Database of protein interaction other than DIP can be used.

Positioning based on the above (4) information on gene pathway means that genes related to intracellular and intercellular information transfer are positioned according to correlations in pathway. FIGURE 8 illustrates the typical pathway, that is, MAPK (mitogen activated protein kinase) pathway. Circles indicate genes, and arrows connecting genes indicated the directions of information transfer between genes. For example, positioning of MEK gene adjacent to Mos gene and positioning Raf gene and ERK gene adjacent to MEK gene demonstrate that these genes belong to the same pathway and genes that transfer information directly are positioned close to each other. Other pathway information, for example, Pathway database (http://www.biocarta.com/), can also be used. Gene positioning can also be reflected on compiled information related to gene relationship, such as metabolic pathway database KEGG (http://www.kegg.kyoto.u.ad.jp).

In this application, gene positioning on substrate on which DNA chips are fixed can be decided according to gene functions (Classification No. 1)

using ontology in PubGene database, and gene positioning within fixation regions (Classification No. 2) can be decided based on gene correlations obtainable by searching PubGene database. However, the contents of PubGene database change, as information contained in literatures keeps increasing yearly. Consequently, gene correlation scores are expected to change, every time new findings appear. Accordingly, gene positionings on the fixation substrate have to change based on the content of information in literature. The positioning of DNA chips on the fixation substrate can be decided using, aside from PubGene, any or the combination of the following; gene interaction database based on experimental results, such as the above DIP, signal transfer pathway database, and metabolic pathway database. Furthermore, database describing gene interaction that will be newly constructed in the future.

FIGURE 15 illustrates an outline of DNA chip making using gene positioning in Bioinformatics. FIGURE 16 is a flow chart of the above. First, gene information is obtained from public database (37) through networking such as Internet or in-house database (38). Using methods published in this Specification based on obtained gene information, positioning of DNA probes (21) on the support medium (23) is decided. Positioning is processed, for example, by a computer (39) connected to networking. Positioning of DNA

probes (21) on the support medium (23) is carried out, for example, using a spotter (30). The positioning of DNA probes (21) on a 96- or 384-well plate (42) that houses DNA probes for spotting is calculated backwards based on performance rules of the spotter (30) so that the previously decided positioning of DNA probes (21) on the support medium (23) is realized. If DNA probes are stocked in other plates (40), the DNA probes are transferred to the above plate (42) using a subdividing robot (41). The subdivision on the plate (42) using a robot (41) is carried out to meet the positioning of probes for spotting that is calculated to realize the previously decided positioning of probes is realized on the support medium. Finally, using the spotter (30), DNA probes (21) housed in the plate (42) are spotted on the support medium (23) to make DNA chips.

2. Positioning methods of gene groups based on experimental data

The above 1, demonstrates concrete examples of gene positioning on DNA chip fixation substrate using Bioinformatics and not based on experimental data. In this paragraph, gene positioning methods are described based on experimental data.

1) Data assembling by chips or FDD

First, 2 kinds of specimens are collected for comparison, and RNA is extracted from each specimen. Two kinds of specimens for comparison consist of, for example, specimens from patients with some disease and

those from healthy persons. Specimens can be any of tissues, blood and cells that contain RNA. It is desirable for the consideration of individual differences to collect plural numbers of specimens, or as many as possible, from both patients and healthy persons. Gene expression in specimens from both subjects is analyzed using DNA chips or FDD. The DNA chip can be, for example, cDNA chips that uses as a probe the PCR-amplified DNA fragments using cDNA clone as template, or can be oligo chips that are used by Aphimetrics Co. in the USA. It is desirable to have gene probes of DNA chips as many as possible for the utmost analysis of the state of gene expression. For example, human genes are thought to number 30,000 to 40,000 and the transcription products to total approximately 100,000 including alternative splicings. Therefore, it is ideal to use DNA chips loaded with several tens of thousands of gene probes. If it is not possible to use DNA chips with a large number of gene probes, the state of gene expression can be analyzed, for example, in transcription products using FDD.

FIGURE 17 illustrate an outline of DNA chip making using gene positioning based on experimental data. FIGURE 18 is a flow chart of FIGURE 17. First, experimental data are assembled in FDD method or DNA chip method, and then, analyzed by a computer (47) to obtain gene information. Based on the obtained gene information, the positioning of DNA probes (21) on the

support medium (23) is decided using the published methods in the Specification of this application. Processes following decision of the positioning are the same as those in DNA chip making using Bioinformatics illustrated in FIGURES 15 and 16.

2) Gene positioning based on statistical analysis

This paragraph describes methods of positioning of DNA chips on the fixation substrate in the Specification of this application, which are based on the results of measurement of the state of expression in 2 kinds of comparable specimens using DNA chip method or FDD method described above. When each of 2 kinds of specimens are plural, results of measurement are statistically analyzed and used for positioning of genes on the fixation substrate. Original data obtained in DNA chip experiments comprise the signal intensity of the 2 kinds of comparable specimens and ratios between the signal intensity of the 2 kinds of specimens. For example, when specimen 1 is labeled with fluorescent dye Cy3 and specimen 2 with Cy5, data obtained are Cy3 fluorescent intensity originated from specimen 1, Cy5 fluorescent intensity originated from specimen 2, and Cy3/Cy5, the ratio of fluorescent intensity.

Original data obtained in FDD experiments comprise the intensity of bands of lanes in electrophoresis of specimen 1, that of specimen 2, and the ratios between the intensities of bands derived from 2

specimens. For example, when both specimens 1 and 2 are labeled with the same dye (Cy3, for example), data obtained are Cy3 fluorescent intensity originating from specimen 1, that originated from specimen 2, and the ratio between 2 fluorescent intensities. Statistical analysis is conducted using (1) fluorescent intensity ratios or (2) fluorescent intensity originated from specimens 1 and 2.

TABLE 39 shows results of experiments using 2 kinds of specimens that are analyzed based on the above (1) fluorescent intensity ratios. Columns in TABLE 39 are, from the left, gene name (symbolic name in Unigene), mean fluorescent intensity ratios, standard deviation (SD) and CV value (SD/mean). In TABLE 39, specimen 1 is CD3+ cell (T cell) originating from peripheral blood of 3 healthy subjects, and specimen 2 is CD3- cell (lymphocytes other than T cell) originating from peripheral blood of 3 healthy subjects. Gene groups in specimens 1 and 2, the fluorescent intensity ratio of which is 3 or higher in the state of expression, are listed in the order of the mean value. TABLES 39 shows results of experiments using DNA chips with several thousands genes. Therefore, similar values can be obtained from other several thousands genes aside from those in TABLE 39, and these genes can be listed in the ascending or descending order of mean values, as one pleases. In TABLE 39, the fluorescent intensity ratios in the above (1) are those of CD3-

cells/CD+ cells, and in (2) are those of CD+ cells/CD- cells. When DNA chips are newly created, the whole or part of several thousand gene probes can be positioned on the DNA chip fixation substrate according to the ascending or descending order of the mean fluorescent intensity. For example, probes can be positioned selecting genes among several thousand genes with the fluorescent intensity ratio 2 or higher, that is, the difference in gene expression between specimens 1 and 2 is twice or more.

FIGURE 5 illustrates an example of the positioning rule. Darkness of color is in proportion to the size of the mean fluorescent intensity ratio. FIGURE 5(A) illustrates an example in which probes are positioned diagonally originating at the corner of DNA chip substrate. FIGURE 5(B) illustrates an example in which probes are positioned concentrically originating at the center of DNA chip substrate. FIGURE 6 illustrates examples in which positionings shown in FIGURE 5 are placed side by side, or in plural numbers, on one DNA chip substrate. FIGURE 6(A) illustrates 4 of the positioning shown in FIGURE 5(A), and FIGURE 6(B) illustrates 4 of the positioning shown in FIGURE 5(B). FIGURE 6 corresponds to gene positioning described in the above 1, in which genes are classified according to functions, and the final positioning is decided based on experimental data.

TABLE 40 shows results of experiments using 2

kinds of specimens same to TABLE 39that are analyzed based on the above (2) specimen 1-originating fluorescent intensity and specimen 2-originating fluorescent intensity. Columns in TABLE 40 are, from the left hand side, gene name (symbolic name in Unigene), t value that is statistic value obtained in t-test, and P value that is significant probability derived from t value. Genes with P value, or significant probability, 0.003 or lower, are listed in the ascending order. TABLE 40 shows the results of experiments using DNA chips with several thousands of genes. Therefore, similar values are obtained from other several thousands of genes aside from those in TABLE 40, and these genes can be listed in the ascending or descending order of t value or P value, as one pleases. When DNA chips are newly created, the whole or part of several thousands of gene probes can be positioned on the DNA chip fixation substrate according to the ascending or descending order of t value or P value. Gene probes can be positioned on the DNA chip fixation substrate in the similar way using other statistic values obtained in testing methods other than t test, such as rank sum test.

When DNA chips are newly created, the whole or part of several thousands of gene probes can be positioned on the DNA chip fixation substrate according to the ascending or descending order of t value. For example, suppose the significant probability P is lower

than 0.2, that is, the difference in the gene expression between specimens 1 and 2 is zero, probes can be positioned selecting genes among several thousands of genes with the 20% probability that the supposition is incorrect. Probe positioning can also be decided based on results of FDD in the same process as in TABLES 39 and 40. Aside from statistic analysis, using support vector machine (SVM) algorithm, well known in the field of machine learning, weight matrix factor (wi) corresponding to each gene is obtained and probe positioning can be decided in the ascending or descending order of wi. Probe positioning can be decided using any method, aside from statistic analysis and machine learning, that can rank genes based on experimental data.

As regards effects of stress on the body, various genes related to the nervous, immune and endocrine systems are thought to play roles. Details have been unclear. Therefore, this Inventors investigated changes in gene expression profile in human peripheral blood samples by creating array with a large number of genes/EST as probes and selected genes, the expression of which changed markedly as stress load increased. As the probes of array, 15,000 kinds of genes/EST were purchased from IMAGE Consortium and used to create DNA probes array for screening. Exercise stress and gastric ulcer stress were chosen as typical stress stimulants.

With respect to exercise stress, subjects on

bicycle ergometers received for a continuous 60 minutes the load of approximately 80% (80% VO_2max) in relative value, when the maximal individual oxygen intake (VO_2max, the maximum value of oxygen taken up by blood in unit time) is defined as 100%. When measured in actual subjects, the 80% VO_2max is approximately 180 watts at bicycle ergometer intensity. Pulse rates during exercise were between 150 and 175/min. The lactate threshold (LT) corresponds to approximately 60% VO_2max, and heart rates between 110 and 130/min. Therefore, the exercise load of 80%VO_2max for 60 min was thought to be sufficient intensity as exercise stress load. Peripheral blood 50 cc was collected within 5 min after the completion of exercise. Messenger RNA was extracted from leukocyte and reverse transcribed in prescribed methods for DNA synthesis. At reverse transcription, fluorochrome-labeled DNA was synthesized using dCTP labeled with fluorescent dye Cy-5 (labeled cDNA: exercise stress load). Meanwhile, prior to exercise stress load, peripheral blood 50 cc was collected from the same subjects. Messenger RNA was extracted in the same process and reverse transcribed using Cy-3 labeled dCTP for cDNA synthesis (labeled cDNA: control).

Equivalent weight of labeled cDNA of exercise stress load and that of control were mixed, placed on the above-mentioned DNA probe array for screening, and hybridized under prescribed conditions. After rinsing,

fluorescent intensity at each spot was measured using a laser scanner for evacuation of kinds and levels of genes expressed in cDNA of exercise stress load and that of control. TABLE 1 shows genes that had changes in the level of expression more than twice, when the level of expression was compared between the two. The increases in the level of expression in TABLE 1 are standardized assuming that the levels of expression of housekeeping genes, such as β-actin, HPRT and GAPDH, is stable. The level of expression of these genes is thought to be stable under various stimulations.

Under exercise stress, the increases in the level of expression were observed in genes related to hormones of the hypothalamic-posterior pituitary system such as vasopressin and anginine vasopressin, adrenocorticotropic hormone (ACTH) receptor genes and genes related to glucocorticoids (cortisol). The level of expression also increased in genes related to catecholamine such as monoamine oxidase. In addition, the expression increased in cytokine genes such as interleukin 6 (IL-6), transcription factors such as NF-κB, and HSP70 and HSP90, heat shock proteins. Observed also were changes in proton pump genes, that is, decreases in Ca^{2+}ATPase, and increases in expression of apoptosis related genes called GADD34.

With respect to gastric ulcer stress, messenger RNA was extracted from peripheral blood 50 cc collected from patients with gastric ulcer, and reverse

transcribed in prescribed methods for cDNA synthesis. At reverse transcription, flurochrome-labeled cDNA was synthesized using dCTP labeled with fluorescent dye Cy-5 (labeled cDNA: gastric ulcer stress). Meanwhile, peripheral blood 50 cc was collected from healthy subjects who do not have gastric ulcer. Messenger RNA was extracted and reverse transcribed using Cy-3 labeled-dCTP for cDNA synthesis in the same process. (labeled cDNA: control).

Equivalent weight of labeled cDNA of gastric ulcer stress and that of control were mixed, placed on the above-described DNA probe array for screening and hybridized under prescribed conditions. After rinsing, fluorescent intensity at each spot was measured using a laser scanner for evaluation of kinds and levels of gene expression in cDNA of gastric ulcer stress and that of control. TABLE 2 shows genes that had changes in the level of expression more than twice, when the level of expression was compared between the two. The increases in the level of expression in TABLE 2 are standardized assuming that the level of expression of housekeeping genes, such as β-actin, HPRT and GAPDH, is stable. The level of expression of these genes is thought to be stable under various stimulations.

Under gastric ulcer stress, the increases in the level of expression were observed in genes related to hormones of the hypothalamic-anterior pituitary system such as CRH, and genes related to ACTH and

glucocorticoid. Conversely, there were little changes in the level of expression of genes related to hormones of the hypothalamus-posterior pituitary system such as vasopressin. Observed also were, as in exercise stress, increases in the expression of cytokine genes such as IL-6 and HSP70 and HSP90, heat shock proteins. The expression of ERK6, a signal transfer gene, and JUN, a transcription factor, as well as anti-inflammation related genes such as prostaglandin increased.

The above findings suggested that genes that had more than twice increases in the level of expression, in either exercise stress or gastric ulcer stress, included genes related to corticotropin-releasing hormones (CRH) such as vasopressin and oxytocin, ACTH and adrenocortical hormones such as glucocorticoid, reflecting activation of the pituitary glands and adrenal cortex by excitation of the hypothalamus. Hereinafter, the hypothalamic-pituitary adrenocortical system is called HPA system. Involvement of catecholamine related genes reflected the activation of sympathetic adrenomedullary (SAM) system. Hormones produced by the endocrine system such as HPA system and SAM system were secreted into blood and bound with hormone receptors on blood cells, increasing the expression of G-proteins and intracellular signal transfer related genes, such as adenylatecyclase and NF-κB. Finally, the expression of cytokine gene was

induced. The expression of stress proteins such as heat shock protein increased as a part of stress reaction at cell level. Activation of glucocorticoid receptor by adrenocortical hormones (glucocorticoid) induced apoptosis in the calcium pathway. Changes in expression occurred in the similar gene groups under 2 completely different stresses suggested that it would be useful in analysis of complex system of stress reaction to observe changes in the expression intensity of these gene groups. That is to say, for analysis of degree of stress, DNA array is the most appropriate, on which the necessary but minimal amount of the following genes are fixed; (1) internal and external standard genes for proofreading genes, (2) stress resistant and survival related genes and hormone genes such as HSP, (3) cytokine genes, (4) apoptosis and cell death related genes, (5) anti-inflammation and cell growth inhibition related genes such as glucocorticoid, (6) immune response related transcription factor or signaling molecules, (7) cell injury-inducing cytokine inductive transcription factor or signaling molecules, (8) cell growth inhibition related transcription factor or signaling molecules, and (9) stress response related transcription factor or signaling molecules.

By dividing probe fixation regions on the support medium according to the above classification (1) to (9), persons performing measurements are able to recognize results in patterns. If probe fixation

regions are not divided by gene functions, processes of displaying results are required after fluorescent signals are obtained, which include changes in positions of spots using computer, number plotting and graph display. By classifying probe genes according to functions and positioning said genes on substrate according to functions, persons performing measurements are able to judge instantly the degree of stress just by displaying fluorescent signals on the screen. Thus, simplification of equipment structure and lowering cost can be achieved easily. Proofreading is necessary in order to eliminate manufacturing variations, when plural numbers of array are created. Oligonucleotides for proofreading are called internal and external standard genes for proofreading. An example of internal standard gene for proofreading is housekeeping gene. The housekeeping gene works in coding of structural proteins and enzymes of the energy metabolism system that are necessary for cell survival. The gene is thought to exist in any cell with different differentiation. For example, β-actin, GAPDH, HPRT, α-tubulin, transferrin receptor and ubiquitin are housekeeping genes. As the gene is already present in subjects' samples such as those of leukocyte, the gene can be the internal standard for proofreading. Internal standard means substances that are already present in samples without being added from outside and can be standard at proofreading. External standard

genes for proofreading are gene sequences that are not present in humans but present in plants, microorganisms and insects. For example, Arabidopsis thaliana gene, plasmid DNA, bacteriophage DNA and firefly luciferase gene are external standard. As the gene is not present in subjects' samples such as those of leukocytes, external standard genes at known concentrations are added to samples at the time of measurement to be used as external standard for proofreading. External standard means substances that are not already present in samples and added separately from outside to be standard for proofreading.

Stress related genes are proteins that are induced at the time of stress caused by physical and environmental factors such as heat shock. For example, HSP, a kind of stress protein, expresses when cells are exposed to high temperature. This HSP expresses and increases by not only external stimulation such as exposure to high temperature but also direct injection of denatured protein into cells (Anathan, J. et al. Abnormal proteins serve as eukaryotic stress signals and trigger the activation of heat shock genes. Science, 232, 252-254, 1986). That is to say, the expression of HSP is not induced by the bodily systems such as nervous, endocrine and immune systems, but by changes occurring inside cells. HSP70, a HSP, is known to have the function of inhibition of apoptosis, which is called program cell death (Mosser, D. D. Roles of

the human heat shock protein hsp70 in protection against stress-induced apoptosis. Mol. Cell Biol., 17, 5317-5327, 1997). Apoptosis is a form of cell death that occurs in cells that are exposed to viral infections, oxidation stress, radiation and anticancer drugs. Apoptosis is induced by excessive stress on cells. HSP70 inhibits cell death by providing cells with stress resistance. Cells in which HSP70 expresses are not only continuously resistant to stress that was the direct cause but also resistant to other stresses (cross resistance), suggesting that HSP is the stress reaction processing mechanism that cells possess. It is extremely useful to know degrees of, or increase or decrease in, expression of stress protein, in order to evaluate degrees of stress at the cellular level. More than 30 kinds of stress proteins are known to exist. Therefore, it is desirable to fix approximately 30 or more oligo probes, including stress proteins, on the oligonucleotide array of this Invention. Stress proteins include, for example, HSP27 (small HSP), HSP40 (Hdj1), HSP47, HSP60/HSP10, HSC70, HSP70, mtHSP70, HSP90, HSP100 (GRP95), HSP150 (ORP150), Bip (GRP78) and TriC.

Genes related to cell survival include, aside from stress proteins, for example, cyclin, which regulates cell cycle, cyclin dependent kinase (CDK), CDK inhibitors (CKI) such as cyclin A, cyclin B, cyclin D, cyclin E, CDK1, CDK2, CDK4 and CDK6.

"Hormones" means organic compounds that are produced in endocrine glands, secreted in blood and carried to target organs, where microdose demonstrates specific physiological actions. Typical endocrine systems include (a) HPA system, (b) SAM system, (c) automatic nervous-pancreatic endocrine system, (d) hypothalamic-sympathetic-renin angiotensin system, (e) hypothalamic-posterior pituitary system, and (f) opioid peptide system. Hormone-related genes include, for example, vasopressin (AVP), vasopressin receptor (AVPR), CRH, CRH receptor (CRHR), MC2R, REN, TH, TSHB and TSHR.

"Cytokines" are general names of bioactive peptides that induce cell growth differentiation and are secreted by blood cells. Cytokines differ from hormones in that cytokine works near where they are secreted and blood concentrations of cytokines are equal to or lower than those of hormones. Major cytokines include granulocyte-colony stimulating factor (G-CSF), macrophage-colony stimulating factor (M-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), erythropoietin, thrombopoietin, stem cell factor (SCF), interleukin-1, -2, -3, -4, -5, -6, -7, -8, -9, -10, -11, and -12, tumor necrosis factor (TNF) and interferon.

Most of the genes with functions of inducing cell death due to stress are thought to be apoptosis-related genes, because almost all cell deaths in the

body are those called apoptosis. Pathways where apoptosis occurs include calcium pathway, death signal pathway, ceramid pathway, mitochondria pathway and DNA injury pathway. In calcium pathway, phosphatidyl-inositol-3-phosphate receptor, calmodulin, ALG2 and carpine play roles. In death signal pathway, TNFα, Fas ligand, TRADD, FADD, RAIDD, FADD, RIP, RAIDD, CASP8, CASP1, CASP3, TRAMP and TRAIL are known to play roles. In ceramid pathway, stress-activated protein kinase (SAPK)/Jun terminal-N kinase (JNK) plays a role. In mitochondria pathway, Bcl-2 associated X protein (Bax2), Bcl-2, Bcl-xL, and caspase gene play roles. In DNA injury pathway, p53, p21, p51, p73 and MDM2 genes play roles. Genes related to anti-inflammation such as glucocorticoid and genes related to growth inhibition include cytochrome P450 gene 11B1 (CYP11B1), CYP11B2, CYP17, CYP21A2, glucocorticoid modulatory element binding protein (GMEB), glucocorticoid receptor repression factor (GRLF), myocilin (MYOC), glucocorticoid receptor α (NR3C1), proopiomelanocortin (POMC) and prostaglandin G/H synthase precursor.

Transcription factors and signaling molecules related to immune response, cytokine induction, growth inhibition and stress resistance include, for example, ATF/CREB transcription factor, NF-κB transcription factor, JUN gene and 14-3-3n gene. In most signal transfers, signals are generally transferred in the mechanism that protein is activated by chemical change

of phosphorylation and the activated protein in turn induces phosphorylation of the adjacent protein, and so forth. Signal transfer pathways are called pathways, which are generally differentiated by naming with representative proteins on pathways (Nomenclature is referred to www.biocarta.com) Known are, for example, MAPK (mitogen activated protein kinase), ATM (ataxia telangiectasia mutated), BCR (B cell receptor), CD40 (related to tumor necrosis factor receptor), CXCR4 (related to chemokine receptor), EGF (epidermal growth factor), EPO (erythropoietin), FAS (fatty-acyl-CoA synthase), FcEpsilon (Fc fragment of IgE receptor), IFN (interferon) alpha, IFN (interferon) gamma, IGF-1 (insulin-like growth factor-1), IL (interleukin)-2, -3, -4, -5, -6, and -18, NFκB (nuclear factor κB), NCF (nerve growth factor), p53 , PDGF (platelet derived growth factor), PLC (phospholipase C), SODD (silencer of death domains), TCR (T cell receptor), TGFβ (transforming growth factor β), TNFR1 (tumor necrosis factor receptor 1), TNFR2 (tumor necrosis factor receptor 2), TPO (thrombopoietin), and Wnt (wingless/int-1). By placing genes that work in coding of proteins that are keys of these pathways on array as probes, signal transfer pathways induced by stress stimulation can be identified. In particular, for patients with chronic stress, which is caused due to dysfunction of one of the proteins on the signal transfer pathway, treatment plans can be determined by identifying the site where

signal transfer is interrupted.

Another example of DNA chip is described, in which oligonucleotides are placed in such a way so that the presence or absence of stress can be understood instantly. This example of practice is one of the examples of gene positioning based on experimental data.

One week before and 5 hours after an examination, peripheral blood 10 cc was collected from one person (patient A) who became excessively tense during examination and 5 persons (control A, B, C, D and E) who did not feel much tension during the same examination. Total RNA was extracted from lymphocytes from both groups. Degrees of stress of patient A, who experienced excessive tension and 5 controls were significantly different in tests by interview conducted by a specialist. Tests by interview confirmed that 5 persons who did not feel excessive tension were not in the state of stress. In experiments with DNA chip housing several thousands genes, the state of expression 1 week before examination was compared with that 5 hours after examination in control A to E. The difference in the state of expression was small between the two. Correlation (R^2) between fluorescent intensity before examination and that after examination was 0.94 to 0.97. FIGURE 10(A) shows scatter plot of control A. Correlation of the same sample is 0.99. Therefore, values 0.94 to 0.97 indicate that the state of expres-

sion before examination did not differ greatly from that after examination. The means of fluorescent intensity ratios with several thousands of genes were obtained in control A to E and listed in ascending order. Then, gene probes were positioned originating at the right upper corner of chip substrate toward the left lower corner following the rules of FIGURE 11. Each square of FIGURE 11 is the position where gene probes are fixed. Numbers in squares indicate that genes are positioned following the direction of arrows in FIGURE 11 in the order of size of the fluorescent intensity ratio (Cy5/Cy3). The positioning of FIGURE 11 shows that genes with large Cy5/Cy3 are concentrated and fixed at the right upper portion and genes with large Cy3/Cy5 at the left lower portion. FIGURE 12 shows patterns obtained following measurement of RNA by DNA chip in FIGURE 11 in 5 controls, A to E. In FIGURE 12, the greater the change in the gene expression, or the greater the ratio Cy5/Cy3 or Cy3/Cy5 is, the darker the gene is. FIGURE 12(A) to 12(E) illustrate patterns of control A to E. In all five, the patters are similar, or the right upper and left lower portions have dark circles and the intermediate is light. FIGURE 13 shows the pattern obtained in measurement in patient A using DNAA chip in FIGURE 11. In FIGURE 13, the right upper and left lower portions have fewer dark circles. The differences between the 2 figures are instantly recognizable. The correlation (R^2) between

fluorescent intensity before and after examination in patient A was 0.88 (see FIGURE 10(B)), which is apparently different from that in 5 controls and the differences are demonstrated in FIGURES 12 and 13.

In order to place the oligonucleotides with the sequence of the above-described genes as probes on the array, it is necessary to decide which parts of the gene sequences are the probes. What must be taken into consideration at that time are melting temperature (Tm) and cross hybridization. In order to carry out highly accurate and highly stringent hybridization between DNA fragments fixed on the DNA array and DNA fragments originating from samples, the relationship is important between hybridization temperature (Th) and Tm of fixed DNA fragment. It is necessary that the difference between the Tm of fixed DNA fragments and the Th does not exceed 30°C. Cross hybridization occurs when there is high homology among DNA sequences. Therefore, in order to prevent cross hybridization from occurring, it is necessary that any of fixed DNA fragments and sample-originated DNA fragments have low homology with DNA fragments that do not hybridize originally with fixed DNA fragments. Furthermore, it is desirable that these DNA fragments do not contain portions that have high homology with sequences with mini hair pin structure or repetitive sequence that is known in human genes as Alu sequence. It is also necessary to calculate the homology not only between gene sequences

fixed on one piece of array but also between DNA sequences and gene sequences of species listed on GENBANK etc. It is desirable not to select DNA sequences for fixed DNA fragments that have high homology with DNA sequences of gene groups that are possibly contained in samples to be measured.

DNA fragments to be fixed as probes can be synthesized in PCR reaction using commercially available cDNA library as template. Oligonucleotide array can be created from synthesized DNA fragments by preparing prescribed concentrations (0.1 to 1.0 μG/μL), and spotting using a spotter on slide glasses that are already coated with polylysine or aminosilane. Degrees of stress are studied using the above-described oligonucleotide array in the following procedure. First, peripheral blood samples are collected from several volunteers who do not have stress symptoms, and messenger RNA is extracted from leukocytes. For example, a messenger RNA pool of average healthy people can be obtained by mixing messenger RNA from many persons. This messenger RNA pool is described hereinafter in the Specification of this application as Universal Control. Next, peripheral blood samples are collected from test subjects, and messenger RNA is extracted from leukocytes. With messenger RNA of peripheral blood of test subjects, labeled cDNA is synthesized using Cy5-dCTP in reverse transcription using oligo dT primer. With messenger RNA in Universal

control, labeled cDNA is synthesized using Cy3-dCTP. Test subjects' cDNA (Cy5 labeled) and Universal control cDNA (Cy3 labeled) are mixed and placed on the same, above-described oligonucleotide array for hybridization at prescribed temperature and duration. It is desirable to have hybridization temperature between 45°C and 70°C, and time between 6 and 18 hours. Following hybridization, fluorescent intensity of Cy5 and Cy3 at each site where genes are spotted is measured using a fluorescent scanner and compared for the difference in the level of expression. Extraction of messenger RNA is performed with either monocytes, which account for 3 to 7% of leukocytes, or lymphocytes, which account for 25 to 33%. Analysis can be expected to reflect better the degrees of stress, because the monocyte has capability to differentiate to macrophage, which is an important cell in the natural immune system, and the lymphocyte to T cell and B cell, which are important cells in the acquired immune system. In addition, these leukocytes have difference cell rotation (dynamics) including maturation in bone marrow, retention time in peripheral blood and life duration. Therefore, it is possible to evaluate acute bioresponse using polynuclear leukocytes (neutrophil), short-term reaction using monocytes and relatively long-term bioresponse using lymphocytes.

Below is an example in which changes in degrees of stress in daily activities were studied in

one subject.

The 793 genes (TABLE 3 and TABLE 38) were selected from GENBANK Unigene by way of key words retrieval, etc. based on the rationale described in the above "Summary of the Invention". These genes work in coding of (1) internal and external standard genes for proofreading, (2) stress resistance and survival related genes such as HSP and hormone genes, (3) cytokine genes, (4) apoptosis and cell death related genes, (5) anti-inflammation related genes such as glucocorticoid and cell growth inhibition related genes, (6) immune response related transcription factor and signaling molecules, (7) cell injury inducing cytokine inductive transcription factor and signaling molecules, (8) cell growth inhibition related transcription factor and signaling molecules, and (9) stress response related transcription factor and signaling molecules.

Next, 793 oligonucleotide probes with highly specific and similar Tm were designed following algorithm consisting of the following procedures; 1. Reading of gene sequence files, 2. Input of salt concentrations and experimental conditions at hybridization, 3. Input of length of fixed DNA fragments, 4. Calculation of melting temperature (Tm) of fixed DNA fragments, followed by elimination from lists of candidates of DNA fragments whose melting temperature does not meet a certain range of Tm, 5. Elimination

from the candidate lists of DNA fragments with specific superorganization or repetitive sequences, 6. Elimination from the candidate lists of DNA fragments with high homology with repetitive sequences such as Alu sequence, and 7. Elimination from the candidate lists of DNA fragments with high homology with other gene sequences. Each of the designed 793 sequences were synthesized using an oligonucleotide synthesizer. The total 796 kinds oligonucleotides comprising the above 793 human gene probes and 3 kinds of oligonucleotide sequences that are not present in humans (lambda DNA, pUC18 plasmid DNA and M13mp18DNA) and are added as external standard genes for proofreading were fixed on a glass substrate in the method published below.

First, commercially available slide glasses (manufactured by Gold Seal Brand) were soaked at room temperature for 2 hours in alkaline solution (sodium hydroxide; 50g, distilled water; 150 ml and 95% ethanol; 200 ml). The slide glasses were transferred to distilled water for rinsing three times to remove alkaline solution completely. The rinsed slide glasses were soaked for 1 hour in 10%poly-L-lysine solution (manufactured by Sigma), pulled out of solution and centrifuged at 500 rpm for 1 min in a centrifuge for microtiter plate to remove poly-L-lysine solution. The slide glasses were placed in suction incubator for drying at 40°C for 5 minutes. Amino group was introduced on the slide glasses. The slide glasses

with amino group were soaked for 2 hours in 1 mM GMBS (by PIERCE) dimethyl sulfoxide solution and rinsed with dimethyl sulfoxide. Maleamide group was introduced on the surface of the slide glasses. Using a DNA synthesizer (manufactured by Applied Biosystem, model 394), oligonucleotides to which thiol group was introduced were synthesized, and purified in high performance liquid chromatography (HPLC). Next, 1 μl of 2 μM synthesized purified oligonucleotides, 4 μL of HEPES buffer (N-2-hydroxyethylpiperazine-N, -2-ethane sulfonic acid; 10 mM, pH 6.5), and 5 μl of additive (ethylene glycol) were mixed to make spotting solution. The prepared spotting solution was spotted randomly on slide glasses using a spotter (manufactured by Hitachi Soft, SPB10 2000). The slide glasses were left at room temperature to fix oligonucleotides on slide glasses.

At that time, with the intention that persons performing measurements can instantly recognize and judge results on the array, probes were fixed in the positions that were published in FIGURE 1 or FIGURE 2. Probe positioning was carried out based on the above-described gene classification (1) to (9).

Peripheral blood 50 cc was collected from a test subject who sat up for 3 nights immediately after the sit-up completed. Immediately, messenger RNA was extracted from leukocytes and preserved at -80°C. Peripheral blood 50 cc was collected from the same test subject after a good rest for 1 week. Messenger RNA

was extracted in the same manner. From messenger RNA obtained immediately after sit-up, Cy5-labeld cDNA was synthesized in reverse transcription using Cy5-dCTP. From messenger RNA obtained after good rest, Cy3-labeled cDNA was synthesized in reverse transcription using Cy3-dCTP.

Equivalent weight of Cy5-labed cDNA and Cy3-labeled cDNA were mixed, placed on the above-described oligonucleotide array for hybridization at 62°C for 2 hours. After rinsing, the fluorescent intensity at each spot was measured using a scanner (manufactured by GSI-Lumonics, ScanArray 5000). FIGURE 3 shows an image after measurement. Fixed probes were positioned as shown in FIGURE 2. The greater the ratio of Cy5 fluorescent intensity/Cy3 fluorescent intensity (sit-up/rest) was, the darker the circle was in FIGURE 3. It is known by experience that immune intensity lowers due to loss of sleep. FIGURE 3 demonstrates that many genes related to inflammation and cell death related genes in FIGURE 2 expressed, suggesting that sitting up for 3 nights resulted in acute malaise, inducing the expression of genes in immune system and apoptosis. The expression of part of stress resistance genes such as HSP increased as a part of stress response. Concerning gene groups related to diseases other than stress.

Cancer can be diagnosed by using DNA chips on which genes that play major roles in cancerization,

infiltration and metastasis such as cancer genes, cancer inhibition genes, growth factor, transcription factor, cytokine, apoptosis, cell cycle modulator and DNA repair genes are fixed. Particularly, by positioning at opposites to each other on the support medium the probes that hybridize with cancer genes and probes that hybridize with transcription products of cancer inhibition genes, it will become easier to recognize instantly the correlation between cancer genes and cancer inhibition genes.

Methods of Evaluation

Method for labeling RNA to produce cDNA

From the total RNA or messenger RNA extracted from cells and tissues, cDNA is synthesized in transcription reaction originating at primer such as oligo-dT primer using transcription enzymes. At the DNA synthesis, for example, fluorescent labels are taken up by cDNA by adding to solution deoxynucleotides to which fluorescent dyes such as Cy3-dCTP, Cy3-dUTP, Cy5-dCTP and Cy5-dUTP are bound. By hybridizing the fluorescent-labeled cDNA with probes fixed on the DNA ship substrate, RNA profile of genes can be measured using the level of fluorescence.

When the level of the total RNA or messenger RNA in cells and tissues is low, labeling is performed using RNA amplification. Amplifications include, for example, T7 or SP3 amplification using T7 or SP3 polymerase reaction. In T7 amplification, transcrip-

tion originates at T7dT primer that has T7 sequence and a sequence with several tens of T bases. T7 sequence is present at the terminal of synthesized cDNA in reverse transcription. Synthesis of RNA that is complementary on cDNA and recognizes this T7 sequence is called in vitro transcription using T7. RNA can be amplified several tens to several hundreds times in in vitro transcription. Fluorescent-labeled cDNA can be synthesized using RNA obtained in this RNA amplification in the same method described above as synthesis of cDNA labeled with RNA. By hybridizing this fluorescent-labeled cDNA with probes fixed on the DNA chip substrate, RNA profile of genes can be measured by the level of fluorescence.

Manufacturing Methods of Chip

When oligonucleotide groups are positioned on the DNA chip using a spotter, it is necessary to house beforehand oligonucleotide group in a 96- or 384-well plate. Positioning of wells of the 96- or 384-well plate on coordinates on the DNA chip is determined by how a spotter is set up. When the positioning on the DNA chip is already determined based on Bioinformatics or experimental data as in the Specification of this application, it is necessary to establish the housing positions of oligonucleotide groups on a 96- or 384-well plate according to the establishment of the spotter. Conventionally, the position of oligonucleotide groups on the DNA chip was established

according to the housing position of oligonucleotide groups in a 96-well plate. In the Specification of this application, conversely, the housing position of oligonucleotide groups on a 96-well plate is established according to the position of oligonucleotide groups on the DNA chip.

Methods of display

1. Real display

The value of fluorescent intensity of Cy5 and Cy3 labeling are displayed in quasi-color according to the intensity. In another quasi-color display, red indicates Cy5 labeling and green Cy3 labeling. On quasi-color images, boarder lines that divide plural sections can be overlapped for display. It is possible to convert images in left and right, or top and bottom inversions and rotation. Graphic displays with bars are possible according to the fluorescent intensity. Three-dimensional bar graphs can be displayed corresponding to the probe fixation positions.

2. Virtual display

More than 2 DNA chips can be displayed on one piece. For example, using quasi-colors, the mean value of each probe, the largeness of standard deviation, correlation between one probe and another probe can be displayed in the order of the size of correlation. Repositioning can be displayed based on information of probe positions already registered on computer.

DNA chip making kit

DNA chip making kit can be offered, which is not a completed DNA kit but a partially completed one. For example, as shown in FIGURE 9, a kit containing a set consisting of substrate for DNA fixation, basic probe set, positioning information on basic probe set, spotter and computer can be offered. Because of being partially completed, in addition to the basic probe set offered as a kit, new probes can be added as the user desires. The user inputs information on gene functions and the state of expression of added probes. Thus, classification of gene functions and the state of expression housed in positioning information of the basic probe set merge to classification of gene functions and the state of expression of added probe set. Real display and virtual display are materialized on computer screen based on the merged classification of gene functions and the state of expression.

As described above, degrees of stress can be evaluated by using the array of this Invention. It is thought that various changes in and close interaction among the three systems or the nervous, endocrine and immune systems lead to complex stress reaction. Conventional methods of measurement of specific hormones in blood are only measuring the endocrine system, but ignoring the interactions among the three, the nervous, endocrine and immune systems. Consequently, it is difficult to find the correlation

between hormone level and degrees of stress in conventional methods because of the individual differences in hormone level and other reasons. In view of defects of conventional methods, this Invention took notice of not only changes in each of the nervous, endocrine and immune systems but also interactions among the three systems, particularly the balance in the interactions. Thus, this Invention was achieved.

It should be further understood by those skilled in the art that the foregoing description has been made on embodiments of the invention and that various changes and modifications may be made in the invention without departing from the spirit of the invention and the scope of the appended claims.

For example, other aspects of this invention are as follows:

(11) A method of displaying results of label detection of hybridization wherein labeled cell-derived RNA are hybridized to an oligonucleotide array comprising multiple subblock regions and oligonucleotides with different base sequences positioned to each of said multiple subblock regions, wherein said oligonucleotides are positioned according to an arrangement pattern wherein oligonucleotides with a first correlation degree are positioned closer to each other than oligonucleotides that have a lower correlation degree; and results of label detection of said hybridization are displayed.

(12) A method of displaying results of label detection of hybridization wherein labeled cell-derived RNA are hybridized to an oligonucleotide array comprising multiple subblock regions and oligonucleotides with different base sequences positioned to each of said multiple subblock regions, wherein said oligonucleotides are positioned according to an arrangement pattern wherein oligonucleotides with a first correlation degree are positioned closer to each other than oligonucleotides that have a lower correlation degree; and results of label detection of said hybridization are rearranged on a screen with determined correlation between oligonucleotides.

(13) A kit for fabrication of an oligonucleotide array comprising multiple subblock regions and oligonucleotides with different base sequences positioned to each of said multiple subblock regions, wherein said oligonucleotides are positioned according to an arrangement pattern wherein oligonucleotides with a first correlation value are positioned closer to each other than oligonucleotides that have a lower correlation value, wherein said kit comprises an oligonucleotide fixation substrate, fixation probes, probe positioning information, a spotter to spot said probes, a monitor screen to display addressing information of the spotter and detection results, or a computer with a monitor that determined the correlation value are provided.

Table 1

GenkBank	Name of gene
M14758	P-glycoprotein (PGY1) mRNA (MDR1)
M25647	vasopressin mRNA; Arginine vasopressin
NM_000707	arginine vasopressin receptor 1B
Z11687	antidiuretic hormone receptor
NM_001402	eukaryotic translation elongation factor 1 alpha 1
U83981	Homo sapiens apoptosis associated protein (GADD34)
NM_006582	glucocorticoid modulatory element binding protein 1
AB034989	KIAA0025 gene product
M69177	Human monoamine oxidase B
J04027	ATPase, Ca++ transporting, plasma membrane 1
NM_002415	macrophage migration inhibitory factor
NM_000261	Homo sapiens myocilin
M14584	Human interleukin 6 mRNA
NM_001078	Homo sapiens vascular cell adhesion molecule 1
NM_005345	heat shock 70kD protein 1
M58603	Human nuclear factor kappa-B DNA binding subunit p105
M34664	Heat shock 60kD protein 1
AF028832	Heat shock 90kD protein 1, alpha

Table 2

GenkBank	Name of gene
AF022224	Bcl-2-binding protein
NM_004244	CD163 antigen
U82812	scavenger receptor cysteine rich Sp alpha
U47741	CREB-binding protein
X58022	corticotropin-releasing factor binding protein
NM_001402	eukaryotic translation elongation factor 1 alpha 1
NM_000862	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1
NM_002228	v-jun avian sarcoma virus 17 oncogene homolog (JUN) mRNA
M14584	Human interleukin 6 mRNA
X79483	ERK6 mRNA for extracellular signal regulated kinase
NM_000529	melanocortin 2 receptor (adrenocorticotropic hormone)
NM_001043	solute carrier family 6 member 2 (SLC6A2)
M59979	prostaglandin G/H synthase 1 precursor
X54079	Heat shock 27kD protein 1
D90224	glycoprotein 34 (gp34)
NM_005345	heat shock 70kD protein 1
AF028832	Heat shock 90kD protein 1, alpha

Table 3

M14758	Homo sapiens P-glycoprotein (PGY1) mRNA (MDR1)
M14752	V-abl Abelson murine leukemia viral oncogene homolog 1
NM_000789	Homo sapiens dipeptidyl carboxypeptidase 1 (angiotensin I converting enzyme) (ACE)
X00351	cytoplasmic beta-actin (ACTB)
L17075	Human TGF-b superfamily receptor type I mRNA; activin receptor-like kinase 1 (ACVRL1; ALK1)
U92649	Homo sapiens snake venom-like protease (cSVP) mRNA, A disintegrin and metalloproteinase domain 17 (tumor necrosis factor, alpha, converting enzyme)
L05500	Homo sapiens adenylate cyclase 1 (ADCY1); Human fetal brain adenylyl cyclase mRNA, 3' end
AF070583	Homo sapiens clone 24648 adenylyl cyclase mRNA, partial cds
NM_004036	Homo sapiens adenylate cyclase 3 (ADCY3)
AF250226	Homo sapiens adenylyl cyclase type VI mRNA
NM_001114	Homo sapiens adenylate cyclase 7 (ADCY7)
Z35309	H.sapiens mRNA for adenylyl cyclase
NM_001116	Homo sapiens adenylate cyclase 9 (ADCY9)
NM_001117	Homo sapiens adenylate cyclase activating polypeptide 1 (pituitary) (ADCYAP1)
NM_001118	Homo sapiens adenylate cyclase activating polypeptide 1 (pituitary) receptor type I (ADCYAP1R1)
M18112	Human poly(ADP-ribose) polymerase mRNA (ADPRT), PARP
M87290	Human angiotensin II type 1 receptor mRNA
X65699	H.sapiens mRNA for angiotensin II receptor
NM_000686	Homo sapiens angiotensin receptor 2 (AGTR2)
NM_005161	Homo sapiens angiotensin receptor-like 1 (AGTRL1)
NM_005162	Homo sapiens angiotensin receptor-like 2 (AGTRL2)
NM_003488	Homo sapiens A kinase (PRKA) anchor protein 1 (AKAP1)
NM_007202	Homo sapiens A kinase (PRKA) anchor protein 10 (AKAP10)
AB014529	A kinase (PRKA) anchor protein 11 (AKAP11); Homo sapiens mRNA for KIAA0629 protein, partial cds
NM_005100	Homo sapiens A kinase (PRKA) anchor protein (gravin) 12 (AKAP12)
NM_007203	Homo sapiens A kinase (PRKA) anchor protein 2 (AKAP2)
NM_006422	Homo sapiens A kinase (PRKA) anchor protein 3 (AKAP3)
NM_003886	Homo sapiens A kinase (PRKA) anchor protein 4 (AKAP4)
NM_004857	Homo sapiens A kinase (PRKA) anchor protein 5 (AKAP5)

Table 4

NM_004274	Homo sapiens A kinase (PRKA) anchor protein 6 (AKAP6)
NM_016377	Homo sapiens A kinase (PRKA) anchor protein 7 (AKAP7)
NM_005858	Homo sapiens A kinase (PRKA) anchor protein 8 (AKAP8)
NM_005751	Homo sapiens A kinase (PRKA) anchor protein (yotiao) 9 (AKAP9)
M63167	Human rac protein kinase alpha mRNA (akt1), complete cds
NM_001283	Homo sapiens AP1S1adaptor-related protein complex 1, sigma 1 subunit (AP1S1)
NM_003916	Homo sapiens adaptor-related protein complex 1, sigma 2 subunit (AP1S2)
AF013263	Homo sapiens apoptotic protease activating factor 1 (Apaf-1) mRNA, complete cds
M74088	adenomatous polyposis coli protein (APC protein); DP2.5
AB023421	Homo sapiens mRNA for heat shock protein apg-1; Heat shock protein (hsp110 family)
U45879	Human inhibitor of apoptosis protein 2 mRNA; Apoptosis inhibitor 1
U45878	Human inhibitor of apoptosis protein 1 mRNA; Apoptosis inhibitor 2
X06820	H.sapiens rhoB gene mRNA; Ras homolog gene family, member B
L25081	Homo sapiens GTPase (rhoC) mRNA, complete cds; Ras homolog gene family, member C
X95282	H.sapiens mRNA for Rho8 protein; Ras homolog gene family, member E
X61587	H.sapiens rhoG mRNA for GTPase; Ras homolog gene family, member G (rho G)
U02570	Human CDC42 GTPase-activating protein mRNA, partial cds
X78817	H.sapiens partial C1 mRNA; Rho GTPase activating protein 4
U17032	Human p190-B (p190-B) mRNA, complete cds; Rho GTPase activating protein 5
AF177663	Homo sapiens GTPase-activating protein 6 isoform 4 (ARHGAP6) mRNA, alternatively spliced, complete cds; Rho GTPase activating protein 6
NM_015366	Homo sapiens Rho GTPase activating protein 8 (ARHGAP8), mRNA

Table 5

X69550	H.sapiens mRNA for rho GDP-dissociation Inhibitor 1
L20688	Human GDP-dissociation inhibitor protein (Ly-GDI) mRNA, D4-GDI
U82532	Homo sapiens GDI-dissociation inhibitor RhoGDIgamma mRNA, complete cds; Rho GDP dissociation inhibitor (GDI) gamma
U64105	Human guanine nucleotide exchange factor p115-RhoGEF mRNA, partial cds; Rho guanine nucleotide exchange factor (GEF) 1
Z35227	H.sapiens TTF mRNA for small G protein; Ras homolog gene family, member H
U96750	Homo sapiens putative tumor supressor NOEY2 mRNA; Ras homolog gene family, member I
NM_005171	Homo sapiens activating transcription factor 1 (ATF1)
M31630	Human cyclic AMP response element-binding protein (HB16) mRNA, 3' end
L19871	Human activating transcription factor 3 (ATF3) mRNA
NM_001675	Homo sapiens activating transcription factor 4 (tax-responsive enhancer element B67) (ATF4)
NM_012068	Homo sapiens activating transcription factor 5 (ATF5)
NM_007348	Homo sapiens activating transcription factor 6 (ATF6)
NM_006856	Homo sapiens activating transcription factor 7 (ATF7)
U33841	Human ataxia telangiectasia (ATM) mRNA
M25647	Human vasopressin mRNA; Arginine vasopressin (neurophysin II, antidiuretic hormone, diabetes insipidus, neurohypophyseal)
L25615	Human arginine vasopressin receptor 1 (AVPR1) mRNA, complete cds
NM_000707	Homo sapiens arginine vasopressin receptor 1B (AVPR1B), mRNA
Z11687	H.sapiens mRNA for antidiuretic hormone receptor; Arginine vasopressin receptor 2 (nephrogenic diabetes insipidus)
AF009674	Homo sapiens axin (AXIN1) ,partial cds
NM_004655	Homo sapiens axin 2 (conductin, axil) (AXIN2), mRNA
U66879	Human Bcl-2 binding component 6 (bbc6) mRNA; BAD protein
AF022224	Homo sapiens Bcl-2-binding protein (BAG-1) mRNA
AF111116	Homo sapiens silencer of death domains (SODD) mRNA; BCL2-associated athanogene 4
NM_017450	Homo sapiens BAI1-associated protein 2 (BAIAP2), transcript variant 1, mRNA
U23765	Human bcl2 homologous antagonist/killer (BAK)
L22474	Human Bax beta mRNA, apoptosis regulator bax
U29680	Human A1 protein; BCL-2-related protein A1 (BCL2A1); BFL1 protein

Table 6

Z23115	H.sapiens bcl-xL mRNA; BCL2-like 1
U59747	Human apoptosis regulator bclw; KIAA0271; BCL2L2
U34584	Human Bcl-2 interacting killer (BIK); NBK apoptotic inducer protein; BP4; BIP1
U14680	Human breast and ovarian cancer susceptibility (BRCA1)
X58957	H.sapiens atk mRNA for agammaglobulinaemia tyrosine kinase
Y14153	Homo sapiens mRNA for beta-transducin repeat containing protein (beta-TrCP)
X83703	H.sapiens mRNA for cytokine inducible nuclear protein; Cardiac ankyrin repeat protein
U13699	Human interleukin 1-beta converting enzyme isoform delta (IL1BCE) mRNA
U60519	Human apoptotic cysteine protease Mch4 (Mch4) mRNA, complete cds
U13021	Human positive regulator of programmed cell death ICH-1L (Ich-1) mRNA, complete cds
U13737	Human cysteine protease CPP32 isoform alpha mRNA, complete cds
U28014	Human cysteine protease (ICErel-II) mRNA, complete cds
U28015	Human cysteine protease (ICErel-III) mRNA, complete cds
U20536	Human cysteine protease Mch2 isoform alpha (Mch2) mRNA, complete cds
U37448	Human Mch3 isoform alpha (Mch3) mRNA, complete cds
U60520	Human apoptotic cysteine protease Mch5 isoform alpha (Mch5) mRNA, complete cds
U60521	Human protease proMch6 (Mch6) mRNA, complete cds
U66838	Human cyclin A1 mRNA, complete cds
X51688	Human mRNA for cyclin A; Cyclin A2
M25753	Human cyclin B mRNA, 3' end.; Cyclin B1
AF002822	Human cyclin B2 mRNA, complete cds
M74091	Human cyclin mRNA
M64349	Human G1/S-specific cyclin D1 (CCND1); cyclin PRAD1; bcl-1 oncogene

Table 7

M90813	Human D-type cyclin (CCND2) mRNA, complete cds; cyclin D2
M92287	Homo sapiens cyclin D3 (CCND3) mRNA, complete cds
M73812	Human cyclin E mRNA sequence
U47413	Human cyclin G1 mRNA, complete cds
U47414	Human cyclin G2 mRNA, complete cds
U11791	Human cyclin H mRNA, complete cds
D50310	Human mRNA for cyclin I, complete cds
U28694	Human eosinophil CC chemokine receptor 3 mRNA, complete cds
U54994	Human CC chemokine receptor 5 (CCR5) mRNA, complete cds
NM_004244	Homo sapiens CD163 antigen (CD163)
M14362	Human T-cell surface antigen CD2 (T11) mRNA, complete cds
J02988	Human T-cell-specific homodimer surface protein CD28 mRNA, complete cds
NM_000732	Homo sapiens CD3D antigen, delta polypeptide (TiT3 complex) (CD3D), mRNA
X03884	Human mRNA for T3 epsilon chain (20K) of T-cell receptor (from peripheral blood lymphocytes).
X04145	Human mRNA for T-cell receptor T3 gamma polypeptide, RON alpha
J04132	Human T cell receptor zeta-chain mRNA, complete cds
M12807	Human T-cell surface glycoprotein T4 mRNA, complete cds
M59040	CD44 antigen (homing function and Indian blood group system)
U82812	Human scavenger receptor cysteine rich Sp alpha mRNA
M80462	Human MB-1 mRNA; CD79A antigen (immunoglobulin-associated alpha)
M89957	Human immunoglobulin superfamily member B cell receptor complex cell surface glycoprotein (IGB) mRNA, CD79B
M27533	CD80 antigen (CD28 antigen ligand 1, B7-1 antigen)
U04343	Human CD86 antigen mRNA, complete cds
M12828	Homo sapiens T-cell surface protein T8 mRNA
M36712	Human T lymphocyte surface glycoprotein (CD8-beta) mRNA, complete cds
S72008	hCDC10=CDC10 homolog [human, fetal lung, mRNA, 2314 nt].
U18291	Human CDC16Hs mRNA, complete cds
X05360	Human CDC2 gene involved in Cell Cycle control; CDK1
M81933	Human cdc25A mRNA, complete cds
M81934	Human cdc25B mRNA, complete cds.
M34065	Human cdc25Hs mRNA, complete cds

Table 8

U00001	Human homologue of S. pombe nuc2+ and A. nidulans bimA; Cell division cycle 27
AF067524	Homo sapiens PITSLRE protein kinase beta SV12 isoform (CDC2L2) mRNA, complete cds
M80629	Human cdc2-related protein kinase (CHED) mRNA; Cell division cycle 2-like 5 (cholinesterase-related cell division controller)
L22005	Human ubiquitin conjugating enzyme mRNA, partial cds; Cell division cycle 34
U63131	Human CDC37 homolog mRNA, complete cds
M35543	Human GTP-binding protein (G25K) mRNA, complete cds
AF022109	Homo sapiens HsCdc18p (HsCdc18) mRNA, complete cds
L33264	Homo sapiens CDC2-related protein kinase (PISSLRE) mRNA; Cyclin-dependent kinase (CDC2-like) 10
M68520	Human cdc2-related protein kinase mRNA, complete cds
X66357	H.sapiens mRNA cdk3 for serine/threonine protein kinase
M14505	Human (clone PSK-J3) cyclin-dependent protein kinase mRNA; cyclin-dependent kinase 4 (CDK4)
X66364	H.sapiens mRNA PSSALRE for serine/threonine protein kinase.
X80343	H.sapiens p35 mRNA for regulatory subunit of cdk5 kinase
U34051	Human cyclin-dependent kinase 5 activator isoform p39i mRNA, complete cds.
X66365	H.sapiens mRNA PLSTIRE for serine/threonine protein kinase
X77743	H.sapiens CDK activating kinase mRNA
X85753	Homo sapiens mRNA for CDK8 protein kinase.
L25676	Homo sapiens CDC2-related kinase (PITALRE) mRNA, complete cds
L25610	Homo sapiens cyclin-dependent kinase inhibitor mRNA; melanoma differentiation-associated protein 6 (MDA6); CDK-interacting protein 1 (CIP1); WAF1; p21

Table 9

NM_004064	Homo sapiens cyclin-dependent kinase inhibitor 1B (p27, Kip1), (CDKN1B) mRNA
U22398	Human Cdk-inhibitor p57KIP2 (KIP2) mRNA, complete cds
L27211	Human CDK4-inhibitor (p16-INK4) mRNA; cyclin-dependent kinase 4 inhibitor (CDK4I; CDKN2); multiple tumor suppressor 1 (MTS1); p16
U17075	Human p14-CDK inhibitor mRNA, complete cds.; p15
AF041248	Homo sapiens cyclin-dependent kinase inhibitor (CDKN2C) mRNA, complete cds.; p18
U40343	Human CDK inhibitor p19INK4d mRNA, complete cds; p19
NM_005194	Homo sapiens CCAAT/enhancer binding protein (C/EBP), beta (CEBPB) mRNA; NF-IL6
AF010127	Homo sapiens Casper mRNA; CASP8 and FADD-like apoptosis regulator; I-FLICE
AF016582	checkpoint kinase 1 (CHK1)
AF009225	Homo sapiens IkB kinase alpha subunit (IKK alpha) mRNA, complete cds; IKK1
L29222	Homo sapiens clk1 mRNA; CDC-like kinase 1
L29216	Homo sapiens clk2 mRNA; CDC-like kinase 2
L29220	Homo sapiens clk3 mRNA; CDC-like kinase 3
M58525	Homo sapiens catechol-O-methyltransferase (COMT) mRNA
NM_001873	Homo sapiens carboxypeptidase E (CPE)
Y00816	Complement component (3b/4b) receptor 1, including Knops blood group system; CD35
M26004	Complement component (3d/Epstein Barr virus) receptor 2; CD21
U84388	Human death domain containing protein CRADD mRNA; CASP2 and RIPK1 domain containing adaptor with death domain
NM_004379	Homo sapiens cAMP responsive element binding protein 1 (CREB1)
U47741	Human CREB-binding protein (CBP) mRNA, complete cds
U47741	Human CREB-binding protein (CBP) mRNA, complete cds
NM_000756	Homo sapiens corticotropin releasing hormone (CRH), mRNA.
X58022	Human mRNA for corticotropin-releasing factor binding protein (CRF-BP).
L23332	Human corticotropin releasing factor receptor mRNA
U34587	Human corticotropin-releasing factor receptor 2 mRNA
U33286	Human chromosome segregation gene homolog CAS mRNA, Chromosome segregation 1 (yeast homolog)-like

Table 10

M37435	Human macrophage-specific colony-stimulating factor (CSF-1) mRNA, complete cds
M10663	Human T-cell granulocyte-macrophage colony stimulating factor (GM-CSF) mRNA
M73832	Human GM-CSF receptor (GM-CSF receptor) mRNA, complete cds
M59941	Human GM-CSF receptor beta chain mRNA; IL3R-beta
X03438	Human mRNA for granulocyte colony-stimulating factor (G-CSF).
M59818	Human granulocyte colony-stimulating factor receptor (G-CSFR-1) mRNA, complete cds
NM_001317	Homo sapiens chorionic somatomammotropin hormone 1 (placental lactogen) (CSH1) mRNA
V00573	Human mRNA encoding placental lactogen hormone
L37042	Homo sapiens casein kinase I alpha isoform (CSNK1A1) mRNA
M55265	Human casein kinase II alpha subunit mRNA, complete cds.
M55268	Human casein kinase II alpha' subunit mRNA, complete cds
X16312	Human mRNA for phosvitin/casein kinase II beta subunit
M92934	Human connective tissue growth factor (CTGF)
X87838	H.sapiens mRNA for beta-catenin
U96136	Homo sapiens delta-catenin mRNA, complete cds, Arm
L06797	Human (clone L5) orphan G protein-coupled receptor mRNA, complete cds; Chemokine (C-X-C motif), receptor 4 (fusin)
NM_000497	Homo sapiens cytochrome P450, subfamily XIB (steroid 11-beta-hydroxylase), polypeptide 1 (CYP11B1), mRNA.
NM_000498	Homo sapiens cytochrome P450, subfamily XIB (steroid 11-beta-hydroxylase), polypeptide 2 (CYP11B2) mRNA.
M14564	Human cytochrome P450c17 (steroid 17-alpha-hydroxylase/17,20 lyase) mRNA, complete cds.
M17252	Human cytochrome P450c21 mRNA, 3' end
U18321	Human ionizing radiation resistance conferring protein mRNA; Death associated protein 3
X76104	H.sapiens DAP-kinase mRNA
AF015956	Homo sapiens Fas-binding protein Daxx mRNA, complete cds
NM_000787	Dopamine beta-hydroxylase (dopamine beta-monooxygenase)

Table 11

M76180	Dopa decarboxylase (aromatic L-amino acid decarboxylase)
AB029497	Homo sapiens gadd153 mRNA for CHOP alternatively spliced isoform (CASIS)
U91985	Human DNA fragmentation factor-45 mRNA, DFF
AF241254	Homo sapiens angiotensin converting enzyme-like protein mRNA
M60278	Human heparin-binding EGF-like growth factor mRNA (HBEGF); diphtheria toxin receptor (DTR)
X68277	H.sapiens CL 100 mRNA for protein tyrosine phosphatase, Dual specificity phosphatase 1, MKP1
U46461	Human dishevelled homolog (DVL) mRNA, complete cds.
NM_004422	Homo sapiens dishevelled 2 (homologous to Drosophila dsh) (DVL2), mRNA
U49262	Human dishevelled (DVL) mRNA, complete cds
M96577	Homo sapiens (E2F-1) pRB-binding protein mRNA; retinoblastoma-binding protein 3 (RBBP3);
NM_001402	Homo sapiens eukaryotic translation elongation factor 1 alpha 1 (EEF1A1)
X04571	Human mRNA for kidney epidermal growth factor (EGF) precursor; urogastrone
U01877	Human p300 protein mRNA, complete cds
X02157	Human mRNA for fetal erythropoietin
M60459	Human erythropoietin receptor mRNA, complete cds
U24231	Human Fas-associating death domain-containing protein mRNA
AJ271408	Homo sapiens mRNA for Fas-associated factor, FAF1
X06948	Human mRNA for high affinity IgE receptor alpha-subunit (FcERI); Fc fragment of IgE, high affinity I, receptor for; alpha polypeptide
M33195	Human Fc-epsilon-receptor gamma-chain mRNA; Fc fragment of IgE, high affinity I, receptor for; gamma polypeptide
M28696	Fc fragment of IgG, low affinity IIb, receptor for (CD32)
X51943	acidic fibroblast growth factor (AFGF) + heparin-binding growth factor 1 precursor (HBGF-1);
U67918	Human keratinocyte growth factor 2 mRNA, complete cds
U66199	Human fibroblast growth factor homologous factor 3 (FHF-3) mRNA, complete cds
U66197	Human fibroblast growth factor homologous factor 1 (FHF-1) mRNA, complete cds
U66198	Human fibroblast growth factor homologous factor 2 (FHF-2) mRNA, complete cds

Table 12

U66200	Human fibroblast growth factor homologous factor 4 (FHF-4) mRNA, complete cds
M27968	Human basic fibroblast growth factor (FGF) mRNA (BFGF; FGFB; FGF2)
M17446	Human Kaposi's sarcoma oncogene fibroblast growth factor mRNA, complete cds
M37825	Human fibroblast growth factor-5 (FGF-5) mRNA, complete cds
X63454	Human fibroblast growth factor 6 precursor (FGF6); HBGF6; HST2
M60828	Human keratinocyte growth factor mRNA; fibroblast growth factor 7 (FGF-7)
U36223	Human fibroblast growth factor 8 (FGF8); androgen-induced growth factor precursor (AIGF); HBGF8
D14838	Human mRNA for FGF-9
M34641	Human fibroblast growth factor (FGF) receptor-1 mRNA
M80634	Human keratinocyte growth factor receptor mRNA; fibroblast growth factor receptor 2 (FGFR2)
M58051	Human fibroblast growth factor receptor (FGFR3) mRNA
L03840	Human fibroblast growth factor receptor 4 (FGFR4) mRNA, complete cds.
Y12863	Homo sapiens mRNA for growth factor FIGF; C-fos induced growth factor (VEGF D)
U01134	Human soluble vascular endothelial cell growth factor receptor (sflt) mRNA; vascular endothelial growth factor receptor 1 (VEGFR1);
U02687	Human growth factor receptor tyrosine kinase (STK-1) mRNA; FLK2
X69878	H.sapiens Flt4 mRNA for transmembrane tyrosine kinase; vascular endothelial growth factor receptor 3 precursor (VEGFR3)
X16707	Human fra-1 mRNA; FOS-like antigen-1
NM_005479	Homo sapiens frequently rearranged in advanced T-cell lymphomas (FRAT1) mRNA
NM_000510	Homo sapiens follicle stimulating hormone, beta polypeptide (FSHB)
M65085	Human follicle stimulating hormone receptor mRNA
AB017363	Homo sapiens mRNA for frizzled-1, complete cds
X02492	Human interferon-inducible mRNA fragment (cDNA 6-16).
M32865	Human Ku protein subunit mRNA; Thyroid autoantigen 70kD (Ku antigen)
U83981	Homo sapiens apoptosis associated protein (GADD34) mRNA
M60974	Human growth arrest and DNA-damage-inducible protein (gadd45) mRNA

Table 13

NM_015675	Homo sapiens growth arrest and DNA-damage-inducible, beta (GADD45B)
NM_006705	Homo sapiens growth arrest and DNA-damage-inducible, gamma (GADD45G)
X01677	liver glyceraldehyde 3-phosphate dehydrogenase (GAPDH)
NM_000805	Homo sapiens gastrin (GAS)
J04040	Human glucagon mRNA, complete cds
L20316	Human glucagon receptor mRNA
NM_000515	Homo sapiens growth hormone 1 (GH1)
M38451	Human placenta-specific growth hormone mRNA
NM_000163	Homo sapiens growth hormone receptor (GHR)
NM_000823	Homo sapiens growth hormone releasing hormone receptor (GHRHR)
NM_004122	Homo sapiens growth hormone secretagogue receptor (GHSR)
NM_006582	Homo sapiens glucocorticoid modulatory element binding protein 1 (GMEB1)
NM_012384	Homo sapiens glucocorticoid modulatory element binding protein 2 (GMEB2)
M69013	Human guanine nucleotide-binding regulatory protein (G-y-alpha) mRNA; Guanine nucleotide binding protein (G protein), alpha 11 (Gq class)
L22075	Human guanine nucleotide regulatory protein (G13) mRNA; Guanine nucleotide binding protein (G protein), alpha 13
NM_004297	Homo sapiens guanine nucleotide-binding protein 14 (GNA14) mRNA
M63904	Human G-alpha 16 protein mRNA, complete cds; Guanine nucleotide binding protein (G protein), alpha 15 (Gq class)
X04526	Human liver mRNA for beta-subunit signal transducing proteins Gs/Gi (beta-G); Guanine nucleotide binding protein (G protein), beta polypeptide 1
M16538	Human signal-transducing guanine nucleotide-binding regulatory (G) protein beta subunit mRNA; Guanine nucleotide binding protein (G protein), beta polypeptide 2
M24194	Human MHC protein homologous to chicken B complex protein mRNA; Guanine nucleotide binding protein (G protein), beta polypeptide 2-like 1
M31328	Human guanine nucleotide-binding protein beta-3 subunit mRNA; Guanine nucleotide binding protein (G protein), beta polypeptide 3

Table 14

AF017656	Homo sapiens G protein beta 5 subunit mRNA; Guanine nucleotide binding protein (G protein), beta 5
U31383	Human G protein gamma-10 subunit mRNA; Guanine nucleotide binding protein 10
U31384	Human G protein gamma-11 subunit mRNA; Guanine nucleotide binding protein 11
NM_012202	Homo sapiens guanine nucleotide binding protein (G protein), gamma 3 (GNG3), mRNA
AF052149	Homo sapiens clone 24733 mRNA sequence; Guanine nucleotide binding protein (G protein), gamma 3, linked
U31382	Human G protein gamma-4 subunit mRNA; Guanine nucleotide binding protein 4
AF038955	Homo sapiens G protein gamma 5 subunit mRNA; Guanine nucleotide binding protein (G protein), gamma 5
AB010414	Homo sapiens mRNA for G-protein gamma 7; Guanine nucleotide binding protein (G protein), gamma 7
S62027	transducin gamma subunit; Guanine nucleotide binding protein (G protein), gamma transducing activity polypeptide 1
X01059	Human placenta mRNA for luteinizing hormone releasing hormone precursor (LHRH).
NM_005311	Homo sapiens growth factor receptor-bound protein 10 (GRB10), mRNA
M96995	Homo sapiens epidermal growth factor receptor-binding protein GRB2 (EGFRBP-GRB2) mRNA sequence
M73077	Human glucocorticoid receptor repression factor 1 (GRF-1) mRNA
X12510	Human mRNA for melanoma growth stimulatory activity (MGSA), groucho
X53799	Human mRNA for macrophage inflammatory protein-2alpha (MIP2alpha,; GRO2 oncogene
L33801	Human protein kinase mRNA; glycogen synthase kinase 3 beta (GSK3 beta); tau kinase subunit; factor A
X17644	Human GST1-Hs mRNA for GTP-binding protein; G1 to S phase transition 1
AF250138	Protein kinase H11;Homo sapiens small stress protein-like protein HSP22 mRNA
D49742	Human mRNA for HGF activator like protein (hyaluronan-binding protein 2)
D50405	Human mRNA for RPD3 protein, Histone deacetylase 1
D16431	Human mRNA for hepatoma-derived growth factor, complete cds

Table 15

M60718	Human hepatocyte growth factor mRNA (HGF); scatter factor (SF); hepatopoeitin A
D14012	Human mRNA for hepatocyte growth factor (HGF) activator precursor
U51004	Homo sapiens protein kinase C inhibitor (PKCI-1) mRNA, Histidine triad nucleotide-binding protein
X58536	Human mRNA for HLA class I locus C heavy chain
K01171	Human HLA-DR alpha-chain mRNA; Class II MHC alpha
X02902	Human mRNA for HLA class II DR-beta 1 (Dw14); Class II MHC beta
M11867	Human MHC class II HLA DR5 DR-beta-chain mRNA, complete cds
U40992	Homo sapiens heat shock protein hsp40 homolog mRNA, complete cds; DnaJ-like heat shock protein 40

Table 16

V00530	Human hypoxanthine-guanine phosphoribosyltransferase (HPRT) IMP:pyrophosphate phosphoribosyltransferase
U76376	Homo sapiens activator of apoptosis Hrk (HRK) mRNA; Harakiri, BCL2-interacting protein (contains only BH3 domain)
AF068754	Homo sapiens heat shock factor binding protein 1 HSBP1 mRNA; Heat shock factor binding protein 1
AF088982	Homo sapiens heat shock protein hsp40-3 mRNA; Heat shock cognate 40
NM_000196	Homo sapiens hydroxysteroid (11-beta) dehydrogenase 2 (HSD11B2)
NM_000862	Homo sapiens hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1 (HSD3B1)
M64673	Human heat shock factor 1 (TCF5) mRNA, complete cds; Heat shock transcription factor 1
M65217	Human heat shock factor 2 (HSF2) mRNA, complete cds; Heat shock transcription factor 2
AB007131	Homo sapiens mRNA for HSF2BP; Heat shock transcription factor 2 binding protein
D87673	Homo sapiens mRNA for heat shock transcription factor 4; Heat shock transcription factor 4
X63368	H.sapiens HSJ1 mRNA; Heat shock protein, neuronal DNAJ-like 1
L08069	Human heat shock protein, E. coli DnaJ homologue mRNA, complete cds; Heat shock protein, DNAJ-like 2
AB003333	Molecular cloning, expression and localization of human 105 kDa heat shock protein, hsp105D
NM_006597	Homo sapiens heat shock 70kD protein 10 (HSC71) (HSPA10), mRNA
NM_005345	Homo sapiens heat shock 70kD protein 1 (HSPA1A), mRNA; Heat shock 70kD protein 1
NM_005346	Homo sapiens heat shock 70kD protein 1 (HSPA1B), mRNA
D85730	Homo sapiens HSPA1L mRNA for Heat shock protein 70 testis variant, complete cds; Heat shock 70kD protein-like 1
U56725	Human heat shock protein mRNA, complete cds; Heat shock 70kD protein 2
L12723	Human heat shock protein 70 (hsp70) mRNA; Heat shock 70kD protein 4
X87949	H.sapiens mRNA for BiP protein; Heat shock 70kD protein 5 (glucose-regulated protein, 78kD)
X51758	Human mRNA for heat shock protein HSP70B'; Heat shock 70kD protein 6

Table 17

L15189	Homo sapiens mitochondrial HSP75 mRNA; Heat shock 70kD protein 9B (mortalin-2)
X54079	Human mRNA for heat shock protein HSP27; Heat shock 27kD protein 1
D89617	Homo sapiens mRNA for MKBP; Heat shock 27kD protein 2
U15590	Homo sapiens heat shock 17kD protein 3 (HSPB3) mRNA, complete cds; Heat shock 27kD protein 3
AJ243191	Homo sapiens mRNA for cardiovascular heat shock protein; Heat shock 27kD protein family, member 7 (cardiovascular)
AF028832	Homo sapiens Hsp89-alpha-delta-N mRNA; Heat shock 90kD protein 1, alpha
M16660	Human 90-kDa heat-shock protein gene, cDNA; Heat shock 90kD protein 1, beta
M34664	Heat shock 60kD protein 1 (chaperonin)
U07550	Human chaperonin 10 mRNA; Heat shock 10kD protein 1
D49547	Human mRNA for heat-shock protein 40; Heat shock 40kD protein 1
AF012106	Homo sapiens DnaJ protein (HSPF2) mRNA, complete cds; Heat shock 40kD protein 2
J03132	Human intercellular adhesion molecule-1 (ICAM-1) mRNA, CD54
M91196	Homo sapiens DNA-binding protein mRNA (Interferon consensus sequence binding protein 1)
NM_005531	Homo sapiens interferon, gamma-inducible protein 16 (IFI16) mRNA
X67325	H.sapiens p27 mRNA (interferon, alpha-inducible protein 27)
J03909	Human gamma-interferon-inducible protein (IP-30) mRNA, complete cds
X03557	Human mRNA for 56-KDa protein induced by interferon
AF083470	Homo sapiens interferon induced tetratricopeptide protein IFI60 (IFIT4) mRNA, complete cds
J04164	Human interferon-inducible protein 9-27 mRNA, complete cds
X57351	Human 1-8D gene from interferon-inducible gene family
X57352	Human 1-8U gene from interferon-inducible gene family
V00551	Messenger RNA for human leukocyte (alpha) interferon
V00538	Messenger RNA for human leukocyte (alpha) interferon
V00542	Messenger RNA for human leukocyte (alpha) interferon
M28585	Human leukocyte interferon-alpha mRNA, complete cds, clone pIFN105
M54886	Human interferon-alpha mRNA, complete cds
V00540	Messenger RNA for human leukocyte (alpha) interferon
V00541	Messenger RNA for human leukocyte interferon (one of eight).
V00550	Messenger RNA for human leukocyte (alpha) interferon.

Table 18

J03171	Human interferon-alpha receptor (HuIFN-alpha-Rec) mRNA, complete cds
X77722	H.sapiens mRNA for interferon alpha/beta receptor
V00547	Human messenger RNA for fibroblast (beta) interferon
X13274	Human mRNA for interferon IFN-gamma
J03143	Human interferon-gamma receptor mRNA, complete cds
U05875	Human clone pSK1 interferon gamma receptor accessory factor-1 (AF-1) mRNA, complete cds
X02669	Human mRNA for type 1 interferon-omega 1.
Y08915	Immunoglobulin (CD79A) binding protein 1
X57025	Human IGF-I mRNA for insulin-like growth factor I
X04434	Human mRNA for insulin-like growth factor I receptor
J03242	Human insulin-lke growth factor II mRNA, complete cds
J03528	Human cation-independent mannose 6-phosphate receptor mRNA; insulin-like growth factor II receptor
M31145	Human insulin-like growth factor binding protein mRNA, complete cds
M35410	Human insulin-like growth factor binding protein 2 (IGFBP2) mRNA
M31159	Human growth hormone-dependent insulin-like growth factor-binding protein mRNA, complete cds
M62403	Human insulin-like growth factor binding protein 4 (IGFBP4) mRNA, complete cds
AF055033	Homo sapiens clone 24645 insulin-like growth factor binding protein 5 (IGFBP5) mRNA, complete cds
M62402	Human insulin-like growth factor binding protein 6 (IGFBP6) mRNA, complete cds
S75725	prostacyclin-stimulating factor [human, cultured diploid fibroblastcells, mRNA, 1124 nt].
AF044195	Homo sapiens IkappaB kinase complex associated protein (IKAP) mRNA, complete cds; IKKAP2
AF080158	Homo sapiens IkB kinase-b (IKK-beta) mRNA, IKK2/beta; IKK2
AF074382	Homo sapiens IkB kinase gamma subunit (IKK-gamma) mRNA, NLK
M57627	Human interleukin 10 (IL10) mRNA, complete cds
U00672	Human interleukin-10 receptor mRNA, complete cds
Z17227	Homo sapiens mRNA for transmebrane receptor protein
M57765	Human interleukin 11 mRNA, complete cds

Table 19

Z38102	H.sapiens mRNA for interleukin-11 receptor
M65291	Human natural killer cell stimulatory factor (NKSF) mRNA, complete cds, clone p35
M65290	Human natural killer cell stimulatory factor (NKSF) mRNA, complete cds, clone p40
U03187	Human IL12 receptor component mRNA, complete cds
U64198	Human Il-12 receptor beta2 mRNA, complete cds
L06801	Homo sapiens interleukin 13 mRNA, complete cds
Y09328	H.sapiens mRNA for IL13 receptor alpha-1 chain
U70981	Human interleukin-13 receptor mRNA, complete cds
AF070546	Homo sapiens clone 24607 mRNA sequence
AF031167	Homo sapiens interleukin 15 precursor (IL-15) mRNA, complete cds.
U31628	Human interleukin-15 receptor alpha chain precursor (IL15RA) mRNA, complete cds
M90391	Homo sapiens putative IL-16 protein precursor, mRNA, complete cds
NM_014443	Homo sapiens interleukin 17B (IL17B), mRNA
NM_013278	Homo sapiens interleukin 17C (IL17C), mRNA
U58917	Homo sapiens IL-17 receptor mRNA, complete cds
D49950	Homo sapiens mRNA for interferon-gamma inducing factor(IGIF), complete cds
AB019504	Homo sapiens mRNA for interleukin-18 binding protein, complete cds
U43672	Human putative transmembrane receptor IL-1Rrp mRNA, complete cds
NM_013371	Homo sapiens interleukin 19 (IL19), mRNA
X02531	Human mRNA for interleukin 1-alpha
M15330	Human interleukin 1-beta (IL1B) mRNA, complete cds
M27492	Human interleukin 1 receptor mRNA, complete cds
X59770	H.sapiens IL-1R2 mRNA for type II interleukin-1 receptor, (cell line CB23).
D12763	Homo sapiens mRNA for ST2 protein

Table 20

U49065	Human interleukin-1 receptor-related protein mRNA, complete cds
X53296	H.sapiens mRNA for IRAP
V00564	Human mRNA encoding interleukin-2 (IL-2) a lymphozyte regulatory molecule
X01057	Human mRNA for interleukin-2 receptor
M26062	Human interleukin 2 receptor beta chain (p70-75) mRNA, complete cds
D11086	Human mRNA for interleukin 2 receptor gamma chain
M17115	Human multilineage-colony-stimulating factor mRNA, complete cds
M74782	Human interleukin 3 receptor (hIL-3Ra) mRNA, complete cds
M13982	Human interleukin 4 (IL-4) mRNA, complete cds
X52425	Human IL-4-R mRNA for the interleukin 4 receptor
X04688	Human mRNA for T-cell replacing factor (interleukin-5).
M75914	Human interleukin 5 receptor alpha mRNA, complete cds
M14584	Human interleukin 6 mRNA, complete cds
X12830	Human mRNA for interleukin-6 (IL-6) receptor
M57230	Human membrane glycoprotein gp130 mRNA, Interleukin 6 signal transducer (oncostatin M receptor)
J04156	Human interleukin 7 (IL-7) mRNA, complete cds
M29696	Human interleukin-7 receptor (IL-7) mRNA, complete cds
M17017	Human beta-thromboglobulin-like protein mRNA, complete cds
L19591	Homo sapiens interleukin 8 receptor alpha (IL8RA) mRNA, complete cds
L19593	Homo sapiens interleukin 8 receptor beta (IL8RB) mRNA, complete cds
M30134	Human P40 protein mRNA, complete cds
M84747	Human interleukin 9 receptor mRNA, complete cds.
U58198	Human interleukin enhancer binding factor 3 mRNA
X60787	Human mRNA for transcription factor ILF
U10323	Human nuclear factor NF45 mRNA, complete cds
U10324	Human nuclear factor NF90 mRNA, complete cds
AF001954	Homo sapiens growth inhibitor p33ING1 (ING1) mRNA, complete cds
NM_001564	Homo sapiens inhibitor of growth family, member 1-like (ING1L) mRNA
NM_000207	Homo sapiens insulin (INS), mRNA
NM_005542	Homo sapiens insulin induced gene 1 (INSIG1)
NM_000208	Homo sapiens insulin receptor (INSR), mRNA.
M10051	Human insulin receptor mRNA, complete cds
J05046	Human insulin receptor-related receptor (IRR) mRNA, 3' end
NM_000209	Homo sapiens insulin promoter factor 1, homeodomain transcription factor (IPF1)

Table 21

L76191	Homo sapiens interleukin-1 receptor-associated kinase (IRAK) mRNA, complete cds
AF026273	Homo sapiens interleukin-1 receptor-associated kinase-2 mRNA, complete cds
X14454	Human mRNA for interferon regulatory factor 1
X15949	Human mRNA for interferon regulatory factor-2 (IRF-2).
Z56281	H.sapiens mRNA for interferon regulatory factor 3
U52682	Human lymphocyte specific interferon regulatory factor/interferon regulatory factor 4 (LSIRF/IRF4) mRNA, complete cds
U51127	Human interferon regulatory factor 5 (Humirf5) mRNA, complete cds
AF027292	Homo sapiens interferon regulatory factor 6 (IRF6) mRNA, complete cds
U53830	Homo sapiens interferon regulatory factor 7A mRNA, complete cds
S62539	insulin receptor substrate-1 [human, skeletal muscle, mRNA, 5828 nt].
S62539	insulin receptor substrate-1 [human, skeletal muscle, mRNA, 5828 nt].
NM_003749	Homo sapiens insulin receptor substrate 2 (IRS2)
NM_003604	Homo sapiens insulin receptor substrate 4 (IRS4)
M13755	Human interferon-induced 17-kDa/15-kDa protein mRNA (interferon-stimulated protein, 15 kDa)
U88964	Human HEM45 mRNA, complete cds
M87503	Human IFN-responsive transcription factor subunit mRNA; Interferon-stimulated transcription factor 3, gamma (48kD); p48

Table 22

L12002	Human integrin alpha 4 subunit mRNA, complete cds; Integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4 receptor)
Y00796	Human mRNA for leukocyte-associated molecule-1 alpha subunit (LFA-1 alpha subunit)., CD11a
J03925	Integrin, alpha M (complement component receptor 3, alpha; also known as CD11b (p170), macrophage antigen alpha polypeptide)
X07979	Integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12);
M15395	Human leukocyte adhesion protein (LFA-1/Mac-1/p150,95 family) beta subunit mRNA, CD18
AF049893	Homo sapiens insulin upstream factor 1 (IUF1) mRNA
M64174	Human protein-tyrosine kinase (JAK1) mRNA, Janus kinase 1
AF005216	Homo sapiens receptor-associated tyrosine kinase (JAK2) mRNA, Janus kinase 2
U09607	Human JAK family protein tyrosine kinase (JAK3) mRNA, complete cds
NM_002228	Homo sapiens v-jun avian sarcoma virus 17 oncogene homolog (JUN) mRNA.
K00558	human alpha-tubulin mRNA, complete cds
AF039597	Ku86 autoantigen related protein 1
X61656	H.sapiens mRNA for growth factor receptor tyrosine kinase; Kinase insert domain receptor (a type III receptor tyrosine kinase)
AB034989	KIAA0025 gene product; MMS-inducible gene; Homo sapiens mRNA for stress protein Herp
D23673	Human mRNA, clone HH109 (screened by the monoclonal antibody of insulin receptor substrate-1 (IRS-1)).
M59964	Human stem cell factor mRNA; (SCF); mast cell growth factor (MGF); c-kit ligand (KITLG)
AF036905	Homo sapiens linker for activation of T cells (LAT) mRNA
M36881	Human lymphocyte-specific protein tyrosine kinase (lck) mRNA
NM_000894	Homo sapiens luteinizing hormone beta polypeptide (LHB)
M73746	Homo sapiens lutropin/choriogonadotropin receptor (LHCGR) mRNA
M13451	Human lamin C mRNA, complete cds, Lamin A
M34458	Human lamin B mRNA, complete cds,
M94362	Human lamin B2 (LAMB2) mRNA, partial cds
NM_016103	Homo sapiens GTP-binding protein Sara (LOC51128), mRNA
AF125392	Homo sapiens insulin induced protein 2 mRNA, complete cds
AF119666	Homo sapiens insulin receptor tyrosine kinase substrate mRNA

Table 23

D12614	Human mRNA for lymphotoxin (TNF-beta), complete cds
U77352	Homo sapiens MAP kinase-activating death domain protein (MADD) mRNA
U68018	Human mad protein homolog (hMAD-2) mRNA; JV18-1.MADR2 OR SMAD2
U68019	Homo sapiens mad protein homolog (hMAD-3) mRNA, complete cds
U44378	Human homozygous deletion target in pancreatic carcinoma (DPC4); mothers against dpp homolog 4 (SMAD4)
AF035528	Homo sapiens Smad6 mRNA, complete cds
AF010193	Homo sapiens MAD-related gene SMAD7 (SMAD7) mRNA, complete cds
NM_000240	Homo sapiens monoamine oxidase A (MAOA), nuclear gene encoding mitochondrial protein, mRNA
M69177	Human monoamine oxidase B (MAOB) mRNA, complete cds
L11284	Homosapiens ERK activator kinase (MEK1) mRNA
L11285	Homosapiens ERK activator kinase (MEK2) mRNA
D87116	Human mRNA for MAP kinase kinase 3b ,complete cds, MEK3
U17743	Human JNK activating kinase (JNKK1) mRNA, complete cds; SEK1
U39064	Human MAP kinase kinase 6 mRNA, complete cds; MEK6
AF013588	Homo sapiens mitogen-activated protein kinase kinase 7 (MKK7) mRNA, complete cds
AF042838	Homo sapiens MEK kinase 1 (MEKK1) mRNA, partial cds
Y10256	H.sapiens mRNA for serine/threonine protein kinase, NIK
NM_003188	Homo sapiens mitogen-activated protein kinase kinase kinase 7 (MAP3K7), mRNA, TAK1
AF096300	Homo sapiens HPK/GCK-like kinase HGK mRNA, complete cds
M84489	Human extracellular signal-regulated kinase 2 mRNA; ERK2
U92268	Homo sapiens mitogen activated protein kinase p38-2 mRNA, complete cds
X79483	H.sapiens ERK6 mRNA for extracellular signal regulated kinase
X79483	H.sapiens ERK6 mRNA for extracellular signal regulated kinase
AF004709	Homo sapiens stress-activated protein kinase 4 (SAPK4) mRNA, complete cds

Table 24

L35253	Human p38 mitogen activated protein (MAP) kinase mRNA; cytokine suppressive anti-inflammatory drug binding protein (CSAID binding protein; CSBP); MAX-interacting protein 2 (MXI2)
L35253	Human p38 mitogen activated protein (MAP) kinase mRNA; cytokine suppressive anti-inflammatory drug binding protein (CSAID binding protein; CSBP); MAX-interacting protein 2 (MXI2)
X60188	Human ERK1 mRNA for protein serine/threonine kinase
L26318	Human protein kinase (JNK1) mRNA; SAPK
X60287	H.sapiens max mRNA
NM_000529	Homo sapiens melanocortin 2 receptor (adrenocorticotropic hormone)
M92424	Human homolog of mouse-double-minute 2; p53-associated mdm2 protein
AF007111	MDM2-like p53-binding protein (MDMX)
NM_002415	Homo sapiens macrophage migration inhibitory factor (glycosylation-inhibiting factor) (MIF),
X72755	H.sapiens Humig mRNA
AB014888	Homo sapiens mRNA for MRJ
X70040	H.sapiens RON mRNA for tyrosine kinase; Macrophage stimulating 1 receptor (c-met-related tyrosine kinase)
M30817	Human interferon-induced cellular resistance mediator protein (MxA)mRNA
M30818	Human interferon-induced cellular resistance mediator protein (MxB) mRNA
U70451	Human myleoid differentiation primary response protein MyD88 mRNA, complete cds
NM_000261	Homo sapiens myocilin, trabecular meshwork inducible glucocorticoid response (MYOC)
AF058696	Nijmegen breakage syndrome 1 (nibrin)
U08015	Human NF-ATc mRNA, complete cds
U43341	Human transcription factor NFAT1 isoform B (NFAT1) mRNA, complete cds
L41067	Homo sapiens NF-AT4c mRNA, complete cds
L41066	Homo sapiens NF-AT3 mRNA, complete cds
U26173	Human bZIP protein NF-IL3A (IL3BP1) mRNA, complete cds
M58603	Human nuclear factor kappa-B DNA binding subunit (NF-kappa-B) mRNA, p105
X61498	H.sapiens mRNA for NF-kB subunit (p49/p100)
M69043	Homo sapiens MAD-3 mRNA encoding IkB-like activity, complete cds, IkBalpha
L40407	Homo sapiens thyroid receptor interactor (TRIP9) gene, complete cds

Table 25

U91616	Human I kappa B epsilon (IkBe) mRNA, complete cds
X77909	H.sapiens IKBL mRNA
U16258	Human I kappa BR mRNA, complete cds
U08191	Human R kappa B mRNA, complete cds
X52599	Human mRNA for beta nerve growth factor
M14764	Human nerve growth factor receptor mRNA
D50420	Non-histone chromosome protein 2 (S. cerevisiae)-like 1
U17327	Human neuronal nitric oxide synthase (NOS1) mRNA
U20141	Human inducible nitric oxide synthase mRNA
M93718	Human nitric oxide synthase mRNA (endothelial)
M10901	Human glucocorticoid receptor alpha mRNA, complete cds
L12260	Human recombinant glial growth factor 2 mRNA, complete cds and flanking regions (neuregulin 1)
M86528	Human neurotrophin-4 (NT-4) gene; neurotrophin 5 (neurotrophin 4/5) (NTF5)
U46752	Oxidative stress induced like; Human phosphotyrosine independent ligand p62B B-cell isoform for the Lck SH2 domain mRNA, partial cds
M25650	Human oxytocin mRNA
X64878	H.sapiens mRNA for oxytocin receptor
AF000546	Homo sapiens purinergic receptor P2Y5 mRNA
U24152	Human p21-activated protein kinase (PAK-alpha; PAK1)
U24153	Human p21-activated protein kinase (PAK-gamma; PAK2); PAK65; S6/H4 kinase
U41745	Human PDGF associated protein mRNA (PAP)
NM_002592	Homo sapiens proliferating cell nuclear antigen (PCNA) mRNA
AF100928	Homo sapiens apoptosis-inducing factor AIF mRNA, nuclear gene encoding mitochondrial protein; Programmed cell death 8
X06374	Human platelet-derived growth factor A subunit precursor (PDGFA; PDGF-1)
M21574	Human platelet-derived growth factor receptor alpha (PDGFRA) mRNA; CD140A antigen

Table 26

M21616	Human platelet-derived growth factor receptor mRNA (PDGFRB); CD140B antigen
M28526	Platelet/endothelial cell adhesion molecule (CD31 antigen), neutrophil; CD31
NM_006211	Homo sapiens proenkephalin (PENK), mRNA
X54936	H.sapiens mRNA for placenta growth factor (PlGF).
AF010310	p53 induced protein (Proline oxidase homolog)
Y13367	H.sapiens mRNA for phosphoinositide 3-kinase; Phosphoinositide-3-kinase, class 2, alpha polypeptide
Y11312	H.sapiens mRNA for phosphoinositide 3-kinase, Phosphoinositide-3-kinase, class 2, beta polypeptide
AJ000008	Homo sapiens mRNA for C2 domain containing PI3-kinase, phosphoinositide-3-kinase, class 2, gamma polypeptide
Z46973	H.sapiens mRNA for phosphatidylinositol 3-kinase, Phosphoinositide-3-kinase, class 3
U79143	Human phosphoinositide 3'-hydroxykinase p110-alpha subunit mRNA, Phosphoinositide-3-kinase, catalytic, alpha polypeptide
S67334	phosphatidylinositol 3-kinase p110 beta isoform=110 kda catalytic subunit [human, mRNA Partial, 3213 nt]. Phosphoinositide-3-kinase, catalytic, beta polypeptide
U86453	Human phosphatidylinositol 3-kinase catalytic subunit p110delta mRNAPhosphoinositide-3-kinase, catalytic, delta polypeptide
X83368	H.sapiens mRNA for phosphatidylinositol 3 kinase gamma, Phosphoinositide-3-kinase, catalytic, gamma polypeptide
M61906	Human P13-kinase associated p85, Phosphoinositide-3-kinase, regulatory subunit, polypeptide 1 (p85 alpha)
X80907	H.sapiens mRNA for p85 beta subunit of phosphatidyl-inositol-3-kinase, Phosphoinositide-3-kinase, regulatory subunit, polypeptide 2 (p85 beta)
D88532	Homo sapiens mRNA for p55pik, Phosphoinositide-3-kinase, regulatory subunit, polypeptide 3 (p55, gamma)
Y08991	H.sapiens mRNA for adaptor protein p150, Phosphoinositide-3-kinase, regulatory subunit 4
M72393	Human calcium-dependent phospholipid-binding protein (PLA2) mRNA; Phospholipase A2, group IVA (cytosolic)
NM_003560	Homo sapiens phospholipase A2, group VI (cytosolic, calcium-independent) (PLA2G6)
AF019770	Homo sapiens macrophage inhibitory cytokine-1 (MIC-1) mRNA (prostate differentiation factor)

Table 27

M95678	Homo sapiens phospholipase C-beta-2 mRNA; Phospholipase C, beta 2
Z16411	H.sapiens mRNA encoding phospholipase c; Phospholipase C, beta 3 (phosphatidylinositol-specific)
L41349	Homo sapiens phospholipase C beta 4 (PLCB4) mRNA; Phospholipase C, beta 4
M34667	Human phospholipase C-gamma mRNA, complete cds
X05199	Human mRNA for plasminogen
J03727	Human phenylethanolamine N-methyltransferase mRNA, complete cds
NM_000939	Homo sapiens proopiomelanocortin (adrenocorticotropin/ beta-lipotropin/ alpha-melanocyte stimulating hormone/ beta-melanocyte stimulating hormone/ beta-endorphin) (POMC)
NM_000306	Homo sapiens POU domain, class 1, transcription factor 1 (Pit1, growth hormone factor 1) (POU1F1)
L14778	Human calmodulin-dependent protein phosphatase catalytic subunit (PPP3CA) mRNA, complete cds and alternative exon
M29551	Human calcineurin A2 mRNA;
S46622	calcineurin A catalytic subunit [human, testis, mRNA, 2134 nt]; Protein phosphatase 3 (formerly 2B), catalytic subunit, gamma isoform (calcineurin A gamma)
M28393	Human perforin mRNA, complete cds
X52479	Human PKC alpha mRNA for protein kinase C alpha; Protein kinase C, alpha
AL049654	Novel human mRNA similar to mouse gene PICK1;Protein kinase C, alpha binding protein
X06318	Human mRNA for protein kinase C (PKC) type beta I.; Protein kinase C, beta 1
U48251	Homo sapiens protein kinase C-binding protein RACK7 mRNA, partial cds; Protein kinase C binding protein 1
U48250	Human protein kinase C-binding protein RACK17 mRNA, partial cds; Protein kinase C binding protein 2
D10495	Homo sapiens mRNA for protein kinase C delta-type; Protein kinase C, delta
X65293	H.sapiens mRNA for protein kinase C-Epsilon; Protein kinase C, epsilon
Z15114	H.sapiens mRNA for protein kinase C gamma (partial); Protein kinase C, gamma
M55284	Human protein kinase C-L (PRKCL) mRNA; Protein kinase C, eta
L18964	Human protein kinase C iota isoform (PRKCI) mRNA; Protein kinase C, iota
D26181	Human mRNA for novel protein kinase PKN; Protein kinase C-like 1
U33052	Human lipid-activated, protein kinase PRK2 mRNA; Protein kinase C-like 2
X75756	H.sapiens mRNA for protein kinase C mu; Protein kinase C, mu
AB015982	Homo sapiens EPK2 mRNA for serine/threonine kinase; Protein kinase C, nu

Table 28

L07032	Human protein kinase C theta (PKC) mRNA; Protein kinase C, theta
J03075	Human 80K-H protein (kinase C substrate) mRNA; Protein kinase C substrate 80K-H
Z15108	H.sapiens mRNA for protein kinase C zeta; Protein kinase C, zeta
U47077	Homo sapiens DNA-dependent protein kinase catalytic subunit (DNA-PKcs) mRNA

Table 29

M59979	prostaglandin G/H synthase 1 precursor (PGH synthase 1; PGHS1; PTGS1); cyclooxygenase 1 (COX1)
M90100	prostaglandin G/H synthase 2 precursor (PGH synthase 2; PGHS2; PTGS2); cyclooxygenase 2 (COX2)
D13540	Homo sapiens SH-PTP3 mRNA for protein-tyrosine phosphatase; Protein tyrosine phosphatase, non-receptor type 11; Shp2
D21210	Human mRNA for protein tyrosine phosphatase (PTP-BAS, type 2); Protein tyrosine phosphatase, non-receptor type 13 (APO-1/CD95 (Fas)-associated phosphatase); FAP
X62055	H.sapiens PTP1C mRNA for protein-tyrosine phosphatase 1C.; Protein tyrosine phosphatase, non-receptor type 6; SHP-1
D11327	Human mRNA for protein-tyrosine phosphatase; Protein tyrosine phosphatase, non-receptor type 7, HePTP
Y00062	Human mRNA for T200 leukocyte common antigen (CD45, LC-A).
AF060231	Homo sapiens herpesvirus entry protein C (HVEC) mRNA; Poliovirus receptor-related 1 (herpesvirus entry mediator C; nectin)
M29870	Human ras-related C3 botulinum toxin substrate (rac) mRNA ras-related C3 botulinum toxin substrate 1; p21-rac1; ras-like protein TC25
M29871	Human ras-related C3 botulinum toxin substrate (rac) mRNA; p21-rac2; small G protein
Z75311	RAD50 (S. cerevisiae) homolog
AF029670	RAD51 (S. cerevisiae) homolog C
AF086904	Protein kinase Chk2
M23379	Human GTPase-activating protein ras p21 (RASA) mRNA; GAP
M15400	Human retinoblastoma susceptibility mRNA, complete cds (RB1)
NM_002892	Homo sapiens retinoblastoma-binding protein 1 (RBBP1) mRNA
S66431	RBP2=retinoblastoma binding protein 2 [human, Nalm-6 pre-B cell leukemia, mRNA, 6455 nt].
X74262	Human chromatin assembly factor 1 p48 subunit (CAF1 p48 subunit); retinoblastoma-binding protein 4
X85134	H.sapiens RBQ-3 mRNA
X85133	H.sapiens RBQ-1 mRNA
U35143	Human retinoblastoma-binding protein (RbAp46) mRNA, complete cds
AF043431	Homo sapiens retinoblastoma-interacting protein (RBBP8) mRNA, complete cds

Table 30

AF039564	Homo sapiens retinoblastoma binding protein (RBBP9) mRNA, complete cds.
L14812	Human retinoblastoma related protein (p107) mRNA; Retinoblastoma-like 1
X74594	Human retinoblastoma-like protein 2 (RBL2; RB2); 130-kDa retinoblastoma-associated protein (p130)
L19067	Human NF-kappa-B transcription factor p65 subunit mRNA, complete cds.
M83221	Homo sapiens I-Rel mRNA, complete cds.
NM_000537	Homo sapiens renin (REN)
AF037195	Homo sapiens regulator of G protein signaling RGS14 mRNA, complete cds.
U50062	Homo sapiens RIP protein kinase mRNA, Receptor (TNFRSF)-interacting serine-threonine kinase 1
AF027706	Homo sapiens serine/threonine kinase RICK (RICK) mRNA; RIP2
M63488	Replication protein A1 (70kD)
X56932	H.sapiens mRNA for 23 kD highly basic protein
U14971	Human ribosomal protein S9 mRNA, complete cds
AF020044	Homo sapiens lymphocyte secreted C-type lectin precursor, mRNA, complete cds
M57502	Human secreted protein (I-309) mRNA; Small inducible cytokine A1 (I-309, homologous to mouse Tca-3)
D49372	Human mRNA for eotaxin; Small inducible cytokine subfamily A (Cys-Cys), member 11 (eotaxin)
U59808	Human monocyte chemotactic protein-4 precursor (MCP-4) mRNA; Small inducible cytokine subfamily A (Cys-Cys), member 13
Z49270	H.sapiens mRNA for chemokine HCC-1; Small inducible cytokine subfamily A (Cys-Cys), member 14
AF031587	Homo sapiens MIP-1 delta mRNA; Small inducible cytokine subfamily A (Cys-Cys), member 15
AF039955	Homo sapiens liver CC chemokine-1 precursor (SCYA16) mRNA; Small inducible cytokine subfamily A, member 16
D43767	Human mRNA for chemokine; Small inducible cytokine subfamily A (Cys-Cys), member 17
Y13710	Homo sapiens mRNA for alternative activated macrophage specific CC chemokine 1; Small inducible cytokine subfamily A (Cys-Cys), member 18, pulmonary and activation-regulated
U77180	Human macrophage inflammatory protein 3 beta (MIP-3beta), Small inducible cytokine subfamily A (Cys-Cys), member 19
S71513	monocyte chemoattractant protein-1 [human, mRNA, 739 nt], MCP-1

Table 31

U77035	Human macrophage inflammatory protein 3 alpha (MIP-3a) mRNA; Small inducible cytokine subfamily A (Cys-Cys), member 20
AF001979	Homo sapiens beta chemokine mRNA; Small inducible cytokine subfamily A (Cys-Cys), member 21
U83171	Human macrophage-derived chemokine precursor (MDC) mRNA; Small inducible cytokine subfamily A (Cys-Cys), member 22
U58913	Human chemokine (hmrp-2a) mRNA; small inducible cytokine subfamily A (Cys-Cys), member 23
U85768	Human myeloid progenitor inhibitory factor-1 MPIF-2 mRNA

Table 32

U86358	Human chemokine (TECK) mRNA; Small inducible cytokine subfamily A (Cys-Cys), member 25
AB010447	Homo sapiens mRNA for CC chemokine eotaxin3;Small inducible cytokine subfamily A (Cys-Cys), member 26
AJ243542	Homo sapiens mRNA for CCL27 chemokine, small inducible cytokine subfamily A (Cys-Cys), member 27
M23452	Human macrophage inflammatory protein (G0S19-1) mRNA, Small inducible cytokine subfamily A (Cys-Cys), member 3; Mip-1a
J04130	Human activation (Act-2) mRNA, complete cds
M21121	Human T cell-specific protein (RANTES) mRNA, Small inducible cytokine A5
X72308	Homo sapiens mRNA for monocyte chemotactic protein-3 (MCP-3), Small inducible cytokine A7 (monocyte chemotactic protein 3)
Y10802	H.sapiens mRNA for monocyte chemotactic protein 2
X02530	Human mRNA for gamma-interferon inducible early response gene (with homology to platelet proteins).
AF030514	Homo sapiens interferon stimulated T-cell alpha chemoattractant precursor, mRNA, complete cds
AF073957	Homo sapiens CXC chemokine BRAK mRNA,Small inducible cytokine subfamily B (Cys-X-Cys), member 14
X78686	H.sapiens ENA-78 mRNA; Small inducible cytokine subfamily B (Cys-X-Cys), member 5 (epithelial-derived neutrophil-activating peptide 78)
U81234	Human chemokine alpha 3 (CKA-3) mRNA; small inducible cytokine subfamily B (Cys-X-Cys), member 6 (granulocyte chemotactic protein 2)
D43768	numan mRNA for SCM-1 (single cysteine motif-1); Small inducible cytokine subfamily C, member 1 (lymphotactin)
NM_003175	Homo sapiens small inducible cytokine subfamily C, member 2 (SCYC2), mRNA.
U84487	Human CX3C chemokine precursor, mRNA, alternatively spliced, complete cds
U10117	Human endothelial-monocyte activating polypeptide II mRNA; small inducible cytokine subfamily E, member 1 (endothelial monocyte-activating)
L36033	Human pre-B cell stimulating factor homologue (SDF1b) mRNA, complete cds; Stromal cell-derived factor 1
M30640	selectin E (endothelial adhesion molecule 1)
M25280	selectin L (lymphocyte adhesion molecule 1)

Table 33

M25322	selectin P (granule membrane protein 140kD, antigen CD62)
U02297	selectin P ligand
X68148	H.sapiens SHC mRNA, Src homology 2 domain-containing transforming protein 1
M20747	Human insulin-responsive glucose transporter (GLUT4) mRNA; Solute carrier family 2 (facilitated glucose transporter), member 4
NM_001043	Homo sapiens solute carrier family 6 (neurotransmitter transporter, noradrenalin), member 2 (SLC6A2)
NM_000454	Homo sapiens superoxide dismutase 1, soluble (amyotrophic lateral sclerosis 1 (adult)) (SOD1); Superoxide dismutase 1, soluble (amyotrophic lateral sclerosis 1 (adult))
X07834	Human mRNA for manganese superoxide dismutase; Superoxide dismutase 2, mitochondrial
J02947	Human extracellular-superoxide dismutase (SOD3) mRNA; Superoxide dismutase 3, extracellular
L13858	Human guanine nucleotide exchange factor mRNA, complete cds, SOS1, Sons of Sevenless
M60618	Human nuclear autoantigen (SP-100) mRNA
NM_000582	Homo sapiens secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1) (SPP1)
U83867	Human alpha II spectrin mRNA, Fodrin
J03161	Human serum response factor (SRF) mRNA; Serum response factor (c-fos serum response element-binding transcription factor)
D86640	Homo sapiens mRNA for stac, (src homology three (SH3) and cysteine rich domain)
M97935	Homo sapiens transcription factor ISGF-3 mRNA, complete cds
M97934	Homo sapiens interferon alpha induced transcriptional activator (ISGF-3) mRNA sequence
L29277	Homo sapiens DNA-binding protein (APRF) mRNA, complete cds
L78440	Homo sapiens STAT4 mRNA, complete cds
L41142	Homo sapiens signal transducer and activator of transcription (STAT5) mRNA
U16031	Human transcription factor IL-4 Stat mRNA, complete cds
U04735	Human microsomal stress 70 protein ATPase core (stch) mRNA; Stress 70 protein chaperone, microsome-associated, 60kD
U26424	Human Ste20-like kinase (MST2) mRNA; Serine/threonine kinase 3 (Ste20, yeast homolog)
U60207	Human stress responsive serine/threonine protein kinase Krs-2 mRNA, Serine/threonine kinase 4

Table 34

L28824	Homo sapiens protein tyrosine kinase (Syk) mRNA; Spleen tyrosine kinase
U49928	Homo sapiens TAK1 binding protein (TAB1) mRNA, complete cds
U63830	Human TRAF family member-associated NF-kB activator TANK mRNA, I-TRAF
M57732	Human hepatic nuclear factor 1 (TCF1) mRNA
M83233	Homo sapiens transcription factor (HTF4) mRNA, complete cds
U08336	Human basic helix-loop-helix transcription factor mRNA, complete cds
D89928	Homo sapiens HKL1 mRNA, complete cds

Table 35

NM_007109	Homo sapiens transcription factor 19 (SC1) (TCF19), mRNA
X58840	Human mRNA for variant hepatic nuclear factor 1 (vHNF1), TCF2
U19345	Homo sapiens AR1 (TCF20) mRNA, partial cds
AF047419	Homo sapiens epicardin mRNA, complete cds.
M31523	Human transcription factor (E2A) mRNA, complete cds
NM_003199	Homo sapiens transcription factor 4 (TCF4)
M62810	Human mitochondrial transcription factor 1 mRNA
NM_003202	Homo sapiens transcription factor 7 (T-cell specific, HMG-box) (TCF7) mRNA.
Y11306	Homo sapiens mRNA for hTCF-4
D15050	Human mRNA for transcription factor AREB6; Transcription factor 8 (represses interleukin 2 expression)
D43642	Human YL-1 mRNA for YL-1 protein (nuclear protein with DNA-binding ability), complete cds
AB012124	Homo sapiens TCFL5 mRNA for transcription factor-like 5, complete cds
NM_003212	Homo sapiens teratocarcinoma-derived growth factor 1 (TDGF1) mRNA
L23959	Homo sapiens E2F-related transcription factor (DP-1) mRNA, complete cds.
NM_003227	Homo sapiens transferrin receptor 2 (TFR2), mRNA
X01060	Human mRNA for transferrin receptor
X70340	H.sapiens mRNA for transforming growth factor alpha
X02812	Human transforming growth factor-beta (TGF-beta; TGFB)
M19154	Human transforming growth factor-beta-2 mRNA; glioblastoma -derived T-cell suppressor factor (G-TSF); bsc-1 cell growth inhibitor; polyergin; cetermin
J03241	Human transforming growth factor-beta 3 (TGF-beta3) mRNA, complete cds.
L11695	Human activin receptor-like kinase (ALK-5) mRNA, complete cds
D50683	Homo sapiens mRNA for TGF-betaIIR alpha, complete cds
L07594	Human transforming growth factor-beta type III receptor (TGF-beta) mRNA, complete cds
NM_000360	Homo sapiens tyrosine hydroxylase (TH), mRNA
L33410	Human c-mpl ligand (ML) mRNA, complete cds
NM_006288	Homo sapiens Thy-1 cell surface antigen (THY1), mRNA
U02571	Human tissue inhibitor of metalloproteinase-3 precursor (TIMP-3) mRNA, complete cds

Table 36

U88540	Homo sapiens Toll-like receptor 1 (TLR1) mRNA, complete cds
U88878	Homo sapiens Toll-like receptor 2 (TLR2) mRNA, complete cds
U88879	Homo sapiens Toll-like receptor 3 (TLR3) mRNA, complete cds
U88880	Homo sapiens Toll-like receptor 4 (TLR4) mRNA, complete cds
U88881	Homo sapiens Toll-like receptor 5 (TLR5) mRNA, partial cds.
M10988	Human tumor necrosis factor (TNF) mRNA
M59465	Human tumor necrosis factor alpha inducible protein A20 mRNA complete cds
M31165	Tumor necrosis factor, alpha-induced protein 6
AF016268	Homo sapiens death receptor 5 (DR5) mRNA, Tumor necrosis factor receptor superfamily, member 10b
AF016267	Homo sapiens TRAIL receptor 3 mRNA, complete cds
AF018253	Homo sapiens receptor activator of nuclear factor-kappa B (RANK) mRNA, complete cds
U94332	Human osteoprotegerin (OPG) mRNA, complete cds
U74611	Human Apo-3 mRNA; Tumor necrosis factor receptor superfamily, member 12 (translocating chain-association membrane protein)
NM_001192	Homo sapiens tumor necrosis factor receptor superfamily, member 17 (TNFRSF17), mRNA
X55313	H.sapiens TNF-R mRNA for tumor necrosis factor receptor type 1.
M32315	Human tumor necrosis factor receptor mRNA, TNF R2
X75962	H.sapiens mRNA for OX40 homologue
X60592	Human CDw40 mRNA for nerve growth factor receptor-related B-lymphocyte activation molecule; CD40
X63717	H.sapiens mRNA for APO-1 cell surface antigen, FAS
M83554	H.sapiens lymphocyte activation antigen CD30 mRNA, complete cds
L12964	Human activation dependent T cell mRNA, complete cds
U37518	Human TNF-related apoptosis inducing ligand TRAIL mRNA, complete cds
AF053712	Homo sapiens osteoprotegerin ligand mRNA, complete cds

Table 37

AF039390	Homo sapiens vascular endothelial cell growth inhibitor (VEGI) mRNA, partial cds
D90224	Human mRNA for glycoprotein 34 (gp34).
L07414	Human CD40-ligand mRNA (Tumor necrosis factor (ligand) superfamily, member 5); CD40L
D38122	Human mRNA for Fas ligand, complete cds; FasL
L09753	Homo sapiens CD30 ligand mRNA, complete cds.
U03398	Human receptor 4-1BB ligand mRNA, complete cds
M14695	Human p53 cellular tumor antigen mRNA, complete cds
U58334	Human Bcl2, p53 binding protein Bbp/53BP2 (BBP/53BP2) mRNA
NM_005427	Homo sapiens tumor protein p73 (TP73) mRNA: Human p73 (monoallelically expressed p53-related protein)
X02592	Human mRNA for T-cell receptor alpha chain (TCR-alpha).
L41690	Homo sapiens TNF receptor-1 associated protein (TRADD) mRNA, 3' end of cds
NM_005658	Homo sapiens TNF receptor-associated factor 1 (TRAF1) mRNA.
U12597	Human tumor necrosis factor type 2 receptor associated protein (TRAP3) mRNA, complete cds
NM_003300	Homo sapiens TNF receptor-associated factor 3 (TRAF3) mRNA.
X80200	H.sapiens MLN62 mRNA (TNF receptor-associated factor 4)
AB000509	Homo sapiens mRNA for TRAF5, complete cds
U78798	Human TNF receptor associated factor 6 (TRAF6) mRNA, complete cds
AF043254	Homo sapiens heat shock protein 75 (hsp75) mRNA, partial cds (tumor necrosis factor type 1 receptor associated protein)
M12886	Human T-cell receptor active beta-chain mRNA, complete cds
U35048	Human putative regulatory protein TGF-beta-stimulated clone 22 homolog (TSC22)
NM_000549	Homo sapiens thyroid stimulating hormone, beta (TSHB)
NM_000369	Homo sapiens thyroid stimulating hormone receptor (TSHR)
X54637	Human tyk2 mRNA for non-receptor protein tyrosine kinase; Tyrosine kinase 2
M26880	Human ubiquitin mRNA, complete cds
AF016371	Homo sapiens U-snRNP-associated cyclophilin (USA-CyP) mRNA, complete cds
NM_001078	Homo sapiens vascular cell adhesion molecule 1 (VCAM1)
M32977	Human heparin-binding vascular endothelial growth factor (VEGF) mRNA
U48801	Human vascular endothelial growth factor B precursor (VEGFB)

Table 38

U43142	Human vascular endothelial growth factor related protein VRP mRNA vascular endothelial growth factor C precursor (VEGF-C); FLT4 ligand
U10564	Human CDK tyrosine 15-kinase WEE1Hu (Wee1Hu) mRNA, complete cds.
AF100779	Homo sapiens connective tissue growth factor related protein WISP-1 (WISP1) mRNA, complete cds
AF100780	Homo sapiens connective tissue growth factor related protein WISP-2 (WISP2) mRNA, complete cds.
AF100781	Homo sapiens connective tissue growth factor related protein WISP-3 (WISP3) mRNA, complete cds.
U81787	Human Wnt10B mRNA, complete cds
Y12692	Homo sapiens mRNA for WNT11 gene
X07876	Human mRNA for irp protein (int-1 related protein) Wingless-type MMTV integration site family member 2
Z71621	H.sapiens Wnt-13 mRNA
U53476	Human proto-oncogene Wnt7a mRNA, complete cds.
Y11094	H.sapiens mRNA for WNT-8B protein
L20422	Human 14-3-3n protein mRNA; Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta polypeptide
M86400	Human phospholipase A2 mRNA, complete cds
L05148	Human protein tyrosine kinase related mRNA sequence; Zeta-chain (TCR) associated protein kinase (70 kD)

Table 39(1)

CD3- cell/CD3+ cell			
Name	ave(n=3)	stdev	CV(%)
ABCC3	21.92	8.05	36.73
LYN	14.90	0.09	40.15
PTGS1	14.66	8.94	0.58
CDKN1C	14.00	11.98	85.57
FLT3	13.91	7.16	51.49
FCER1G	13.20	12.96	91.63
CHUK	11.63	3.76	32.30
VEGFC	10.80	4.31	39.92
POLK	10.65	10.14	95.21
AVPR1A	10.22	6.37	62.29
CYP7A1	9.66	2.67	27.70
PRKCBP2	9.62	9.49	98.70
GNG11	8.14	4.24	52.03
GNAZ	8.06	2.92	36.17
AVPR2	7.72	5.35	69.27
CD9	7.62	1.50	19.69
GJB3	7.49	4.21	79.72
DTR	7.39	2.97	40.21
HLA-DRB1	7.31	4.82	81.16
RPC32	7.30	5.82	79.79
NRG1	7.25	2.92	61.00
MAFG	7.19	3.82	29.64
MGST2	6.95	3.36	48.37
RAB13	6.75	2.38	22.94
SLC7A7	6.38	1.63	25.63
CYP1B1	6.36	3.18	50.01
IL6	6.07	2.06	33.91
PDGFA	6.07	2.96	48.81
MYCL1	6.06	3.14	30.99
FES	6.04	4.23	70.04
TNFRSF1B	5.86	3.74	63.82
IPF1	5.79	5.75	99.45

CD3- cell/CD3+ cell			
Name	ave(n=3)	stdev	CV(%)
YWHAH	5.46	1.61	29.41
PIG3	5.31	2.78	67.68
BTK	5.26	2.92	55.53
E2F3	5.00	2.52	50.53
FCGR2B	4.92	1.53	44.29
UGT2B7	4.72	2.70	40.31
ATP1B4	4.66	3.77	81.02
PENK	4.63	0.82	17.65
BAG4	4.60	1.53	85.30
PLA2G4A	4.48	2.87	64.04
TLR4	4.46	0.89	19.95
FGR	4.32	0.93	33.34
ALDH1	4.22	2.58	61.08
NOS1	4.21	2.74	65.02
TLR5	4.14	1.23	51.76
ABCC1	4.09	2.77	78.31
ALDH2	4.08	3.04	65.86
ARHGAP6	4.08	0.86	21.04
IL1R2	3.88	1.88	57.22
SOD2	3.76	0.66	17.57
NR1H4	3.66	1.59	43.29
TCF4	3.65	0.90	95.71
SKIL	3.42	0.71	20.79
IL8RA	3.41	0.72	74.47
POU2F2	3.36	0.77	49.91
CDC25C	3.33	1.34	42.41
PAK1	3.28	1.25	37.96
SLC1A4	3.19	0.69	21.61
SLC1A3	3.15	0.72	22.93
BRAF	3.13	0.07	2.37
ATF3	3.11	0.46	14.66

Table 39(2)

CD3- cell/CD3+ cell			
Name	ave(n=3)	stdev	CV(%)
TRA@	15.08	11.13	73.78
CD3G	12.03	1.36	11.32
CD3E	10.55	1.10	10.39
IL7R	9.77	6.15	62.90
BCL2	9.54	2.88	30.18
PCNA	8.14	4.35	53.41
HSPA10	7.52	3.62	48.14
EPHX2	7.04	2.33	33.07
CD8B1	6.97	3.98	57.03
FYN	6.97	0.97	13.87
STAT1	6.44	3.54	54.87
HSPF1	6.44	0.87	13.55
CCR5	5.63	2.43	43.20
ELF1	5.33	3.42	64.21
NR3C2	5.22	4.78	91.53
TGFBR2	5.01	2.88	57.45
ATRX	4.65	2.49	53.64
HLJ1	4.64	3.41	73.62
CYP2J2	4.58	1.29	28.22
E2F4	4.44	1.96	44.28
STAT4	4.35	4.75	108.99
NFATC3	4.26	2.62	61.62
PIK3R1	4.17	1.23	29.47
PPP3CB	4.12	2.00	48.48
CLK1	4.11	4.04	98.37

CD3- cell/CD3+ cell			
Name	ave(n=3)	stdev	CV(%)
RBL2	3.76	2.16	57.55
KIAA0194	3.75	0.91	24.32
GSTM3	3.75	2.21	58.89
GZMA	3.74	4.32	115.44
CDC25B	3.70	0.50	13.54
KRAS2	3.65	0.98	26.90
ITGA4	3.49	1.62	46.24
IL13RA2	3.48	2.01	57.64
SOD1	3.47	0.21	6.20
CCNG1	3.38	1.25	36.86
PAP	3.30	0.87	26.40
ABCE1	3.27	0.48	14.75
TNFRSF1	3.25	1.01	30.96
CHST5	3.19	2.34	73.37
STAC	3.16	2.62	82.98
ATP1A3	3.14	0.87	27.84
HINT	3.14	1.49	47.46
ABCC5	3.12	1.06	34.01
TAF1B	3.11	1.33	42.84
CD80	3.10	0.20	6.29
CD28	3.10	0.94	30.25
STCH	3.08	0.86	27.91
TTF1	3.07	0.73	23.81
POLR2C	3.05	1.49	48.89
HGF	3.01	1.37	45.36

Table 40

Name of gene	t value	p value
ABCE1	-24.009	0.000071
IFNB1	-16.646	0.000298
BMI1	-15.039	0.000443
KRAS2	-14.382	0.000527
CD80	-14.224	0.000550
IL8RA	13.916	0.000598
BAG4	13.105	0.000754
POLK	13.054	0.000766
NFATC2	-12.400	0.000933
NRG1	12.049	0.001041
TLR5	11.925	0.001083
HGF	-10.946	0.001501
POLI	-10.621	0.001682
CDC25B	-10.463	0.001780
IL6	10.452	0.001787
SELE	-10.449	0.001789
MAX	10.384	0.001832
FCGR2B	10.296	0.001891
COX10	-10.208	0.001953
YWHAH	10.138	0.002005
ADH6	9.976	0.002130
PRKCZ	-9.925	0.002171
AVPR2	9.872	0.002215
GJB3	9.808	0.002269
CLK2	-9.694	0.002371
TRA@	-9.543	0.002514
EPHX2	-9.540	0.002517
CD3G	-9.441	0.002617
MAP2K6	-9.413	0.002646
ALDH1	9.196	0.002886
PCNA	-9.134	0.002959
CD3E	-9.131	0.002962